EXHIBIT 7

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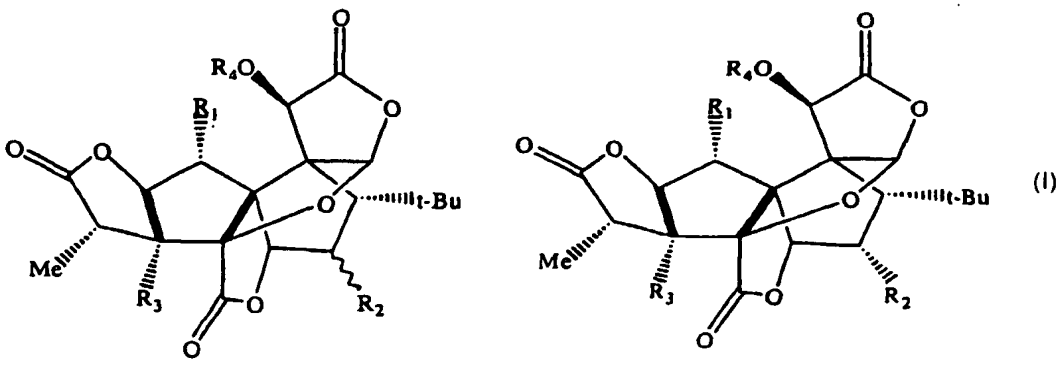
(54) Title: ANALOGS OF TERPENE TRILACTONES FROM GINKGO BILOBA AND RELATED COMPOUNDS AND USES THEREOF



(57) Abstract: The subject invention provides compounds having the structure Formula (I): wherein R_1 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; R_2 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; R_3 is H or OH; R_4 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; and wherein at least one of R_1, R_2, R_3, or R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety, or an optically pure enantiomer of the compound or wherein R1 is H or OH; R2 is H, OH, halogen, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, -N3, -COR5, -CONR5R6, -CO2R5, -OCOR5, -NH(OH), -NR5R6, -NHCOR5, .N(OH)COR5, -CH2OR5, -OCH2CO2R5, -CH2SR5, -CH2NR5R6. -SR5, -OSR5, or -NR5SO2R6, where R5 and R6 are each independently hydrogen substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms; R3 is H or OH; R4 is H, (C1-C10) alkyl, (C1-C10) alkenyl, (C1-C10) alkynyl, -A-Ar, -A-Z-Ar, -SO_2-Ar, or -A-NR_5, or -R_7, where A, Z and Ar are as defined herein, and the use of the compounds for detecting or identifying a receptor which binds the compounds of the invention or for treating a PAF associated condition in a subject.

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Applicants: Koji Nakanashi et al.
Serial No.: 10/579,162
Filed: November 9, 2004
Exhibit 7

ANALOGS OF TERPENE TRILACTONES FROM GINKGO BILOBA AND RELATED COMPOUNDS AND USES THEREOF

This application is a continuation-in-part of U.S. Serial No. 10/109,965, filed March 29, 2002, and claims the benefit of U.S. Provisional Application No. 60/436,916, filed December 27, 2002, the entire contents of both are hereby incorporated by reference.

Throughout this application, various publications are referenced by the first author's last name and the year of publication in parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Background of the Invention

Ginkgo biloba L., the last surviving member of a family of trees (*Ginkgoacea*) that appeared more than 250 million years ago, has been mentioned in the Chinese Materia Medica for more than 2,500 years (Drieu, 2000). A number of *G. biloba* natural products have been isolated (Hasler, 2000), the most unique being the terpene trilactones, i.e. ginkgolides A, B, C, J and M (1-5) and bilobalide (6) (Fig. 1) (Nakanishi, 1967; Okabe, 1967; Nakanishi,, 1971; Weinges, 1987). The ginkgolides are diterpenes with an aesthetic cage skeleton consisting of six 5-membered rings, i.e., a spiro[4.4]nonane carbocycle, three lactones and a tetrahydrofuran. The difference between the five ginkgolides lies in the variation in the number and positions of hydroxyl groups on the spirononane framework (Fig. 1).

A standardized *G. biloba* extract (EGb 761) containing terpene

trilactones (5-7%) and flavonoids (22-24%) has demonstrated neuromodulatory properties (DeFeudis, 2000), and several clinical studies using EGb 761 have reported positive effects on various neurodegenerative diseases (Logani, 2000; Oken, 1998; Kleijnen, 1992; Søholm, 1998; Diamond, 2000; van Dongen, 2000), including Alzheimer's disease (AD). In two studies involving a total of 549 AD patients, EGb 761 significantly slowed the loss of cognitive symptoms of dementia, with an efficacy in between donezepil (Aricept®) and rivastigmine (Exelon®), the two currently marketed drugs for treatment of AD symptoms (Le Bars, 1997; Kanowski, 1996). Moreover, a recent study by Schultz and co-workers found that EGb 761 upregulated several genes in rat hippocampus and cortex, including genes expressing proteins such as transthyretin and neuronal tyrosine/threonine phosphatase, both of which are believed to be involved in AD (Watanabe, 2001). Several recent studies on healthy volunteers have shown positive effects of EGb 761 on short-term working memory (Kennedy, 2000; Polich, 2001; Rigney, 1999; Stough, 2001) indicating that constituents of *G. biloba* also influence the brain under physiological conditions.

Although the molecular basis for the action of *G. biloba* terpene trilactone constituents on the central nervous system (CNS) is only poorly understood, it is known that the ginkgolides, particularly ginkgolide B (GB, **2**), is a potent *in vitro* antagonist of the platelet-activating factor receptor (PAFR) (Braquet, 1985).

A number of *G. biloba* constituents have been isolated, including the unique terpene trilactones, i.e., ginkgolides A, B, C, J and M and bilobalide (Nakanishi, 1967; Okabe, 1967; Nakanishi, 1971; Weinges, 1987). Ginkgolides are diterpenes with a cage skeleton consisting of six 5-membered rings, the difference between the five ginkgolides being in the variation in the number and positions of hydroxyl groups on the spirononane framework.

Although the molecular basis for the action of *G. biloba* terpene trilactone constituents in the central nervous system (CNS) is only poorly understood, it is known that ginkgolides, particularly ginkgolide B (GB, 1, Figure 1), is a potent *in vitro* antagonist of the platelet-activating factor receptor (PAFR) (Braquet, 1987; Braquet, 1991). The PAFR is a potential target for neurodegenerative diseases (Singh, 2001) such as senile dementia (http://www.herbs.org/greenpapers/ginkgo.htm), stroke (Lindsberg,1990) and nerve cell damage due to ischemia (Krieglstein, 1994).

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, Fig. 2) is a phospholipid mediator involved in numerous disorders. PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine)has been suggested as a retrograde messenger in long-term potentiation (LTP) (Kato, 1994), thus indicating the importance of the PAFR as a target for ginkgolides. PAF has been implicated in a number of immunological, inflammatory and vascular disorders (Chung, 1995) including asthma (Nagase, 2002) and endotoxic shock (Tsuneyuki, 1996). In the latter case, PAFR antagonists have been shown to attenuate the effects of endotoxic shock in rats. Hyperacute rejections arising from PAF-associated reactions of either xenoperfusion (Cruzado, 1997) or renal transplants (Grino, 1994) have been found to be preventable by PAFR antagonists. In a separate study, PAFR antagonists significantly prevented pulmonary edema after myocardial ischemia in dogs (Taniguchi, 1992). The role PAF plays in a broad range of physiological conditions appears well-documented given the above examples, underscoring the importance of PAFR antagonists in inhibiting their undesirable effects.

These effects are manifested through binding of PAF to the PAFR, a G protein-coupled receptor that is found in organs such as the lungs, liver, kidneys (Ishii, 2000; Prescott, 2000; Shukla, 1996), and brain (Bito, 1992; Mori, 1996). The function of PAF

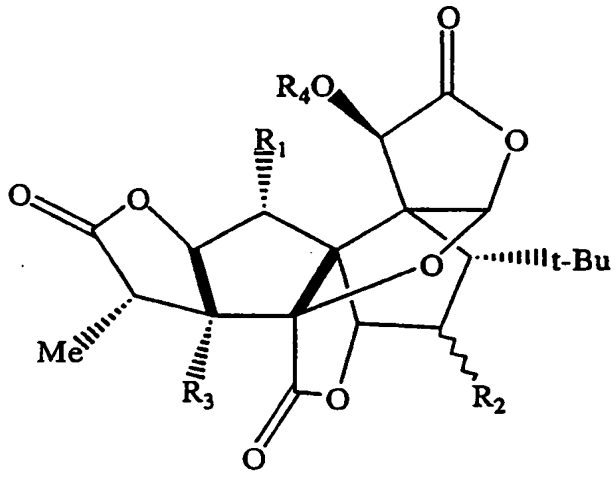
in the brain is still not clear, although PAF has been suggested to play a role in diseases of aging (Kroegel, 1992), and in initiating HIV-related neuropathogenesis (Perry, 1998).

With few exceptions previous structure-activity relationship (SAR) studies of terpene trilactones on the PAFR have focused almost entirely on derivatives of GB (**2**). In all cases the derivatives were evaluated for their ability to prevent PAF-induced aggregation of rabbit platelets. Corey *et al.* investigated various intermediates encountered in the total syntheses of ginkgolide A (GA, **1**) (Corey, 1988), GB (**2**) (Corey, 1988) and bilobalide (BB, **6**) (Corey, 1987), and found that although the terminal methyl-bearing lactone was not essential for activity and could be replaced by other lipophilic groups (Corey, 1989), the *tert.* butyl group was important for PAFR antagonism (Corey, 1991). Park *et al.* synthesized over 200 derivatives of GB (**2**), with particular focus on aromatic substituents at 10-OH, and found most of them to be more potent than the parent compound (Park, 1996). Similar derivatives recently synthesized by Hu *et al.* also yielded compounds more potent than GB (**2**) (Hu, 1999; Hu, 2000), whereas other variations in GA (**1**) and GB (**2**) led to a decrease in activity (Hu, 2000; Hu, 2001).

However, none of the cited references disclose labeled analogs of ginkgolides useful for imaging studies. The following describes the preparation of a series of ginkgolide derivatives with photoactivatable groups and fluorescent groups, as well as groups that potentially can be radiolabeled with positron emitters such as ^{11}C or ^{18}F. These analogs, together with the native terpene trilactones (**1-6**), have been assessed for their ability to displace radioligand binding to cloned PAFR.

Summary of the Invention

The subject invention provides a compound having the structure:



wherein R_1 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

wherein R_2 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

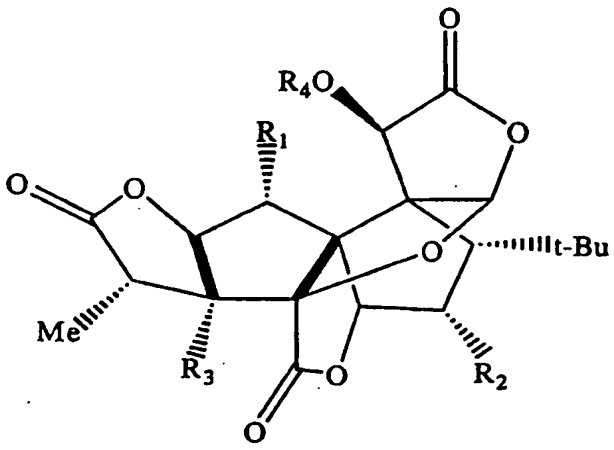
wherein R_3 is H or OH;

wherein R_4 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; and

wherein at least one of R_1, R_2, R_3, or R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety,

or an optically pure enantiomer of the compound.

The invention also provides a compound having the structure:



wherein R_1 is H, OH;

wherein R_2 is H, OH, F, Br, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl;

wherein R_3 is H or OH; and

wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_5, or -R_6,

where A is a (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

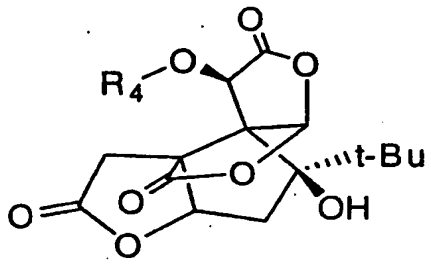
Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an

aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$, $-NHCOR_5$, $-NH(OH)$, $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, a (C_1-C_{10})alkyl or a (C_1-C_3) cycloalkyl group , $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or $-Z-A-Z'-$ in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

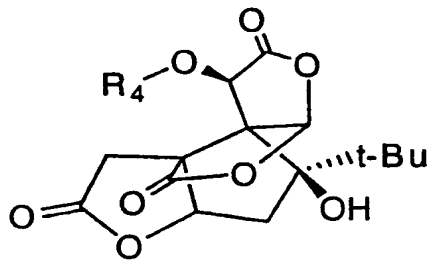
or an optically pure enantiomer or a salt of the compound.

The invention also provides a compound having the structure:



wherein R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety. The photoactivatable moiety, fluorescent moiety, and radioactive moiety are as defined above.

The invention also provides a compound having the structure:



wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2-Ar$, or -A-NR_5, or $-R_6$,

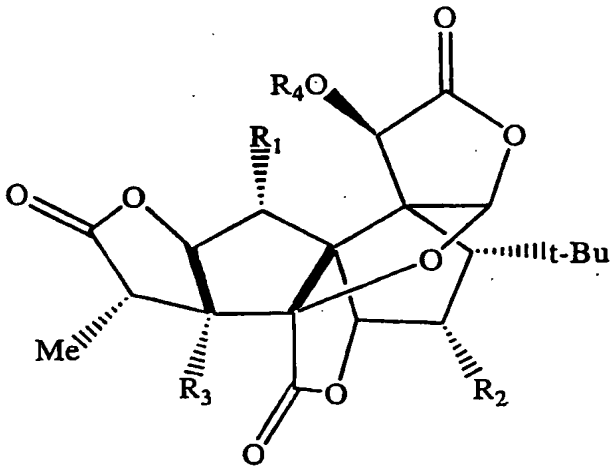
where A is a (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5)alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$, $-NHCOR_5$, $-NH(OH)$, $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, a (C_1-C_{10}) alkyl or a (C_3-C_{10}) cycloalkyl group, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen.

The subject invention also provides a compound having the structure:



wherein R1 is H or OH;

wherein R2 is H, OH, halogen, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, -N3, -COR5, -CONR5R6, -CO2R5, -OCOR5, -NH(OH), -NR5R6, -NHCOR5, -N(OH)COR5, -CH2OR5, -OCH2CO2R5, -CH2SR5, -CH2NR5R6, -SR5, -OSR5, or -NR5SO2R6,

where R5 and R6 are each independently hydrogen, substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms;

wherein R3 is H or OH;

wherein R4 is H, (C1-C10) alkyl, (C1-C10) alkenyl, (C1-C10) alkynyl, -A-Ar, -A-Z-Ar, -SO_2-Ar, or -A-NR_5, or -R_7,

where A is (C1-C8) alkyl, (C2-C8) alkenyl, (C2-C8) alkynyl, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, substituted or unsubstituted (C1-C10) alkyl, (C2-C10) alkenyl, (C2-C10) alkynyl, (C1-C10) haloalkyl, (C1-C10) alkoxy, (C2-C10) alkenyloxy group, (C2-C10) alkynyloxy, (C1-C10) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, -COR_6, -$CONR_6R_6$, -CO_2R_6, -$NHCOR_6$, -NH(OH), -N(OH)COR_6, -$CHOR_6$, -$OCH_2CO_2R_6$, -CH_2SR_6, -$CH_2NR_6R_6$, -SR_6, -OSR_6, -NR_6R_6, -$NR_6SO_2R_6$,

where R6 is hydrogen, (C1-C10) alkyl, (C3-C10) cycloalkyl, -SCX3 in which X is a halogen, -CN, -NO2 or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur or nitrogen,

or an optically pure enantiomer, or a tautomer, or a salt of the compound.

The invention further provides a method of inhibiting activation of the platelet-activating factor receptor (PAFR) which comprises contacting the PAFR with any of the disclosed compounds so as to thereby inhibit activation of the PAFR.

The invention further provides a method of treating a pletelet-activating factor (PAF) associated condition in a subject comprising administering to the subject an amount of any of the disclosed compounds effective to treat the PAF-associated disease.

The invention further provides a process for synthesizing compounds of the invention.

The invention also provides a process of forming a secondary amine compound from an azide of the compound by contacting the azide of the compound with hydrogen, palladium and carbon in a polar-protic solvent.

The invention also provides a method of detecting the localization of a receptor that binds any of the described compounds in a subject, comprising administering the compound to the subject and imaging the subject's body to identify the point of accumulation of the compound in the subject, thereby detecting the localization of the receptor in the subject.

The invention also provides a method of identifying a receptor that binds any of the described compounds in a subject, comprising administering the compound to the subject, imaging the subject's body to identify the point of accumulation of the compound in the subject, and identifying the receptor present at the point of accumulation of the compound, thereby

identifying the receptor in the subject.

The terpene trilactones, ginkgolides and bilobalide, are structurally unique constituents of *Ginkgo biloba* extracts, which exhibit various neuromodulatory properties. Although the terpene trilactones are believed to be responsible for some of these effects, the specific interactions with targets in the central nervous system remain to be elucidated on a molecular level. Ginkgolides are known antagonists of the platelet-activating factor (PAF) receptor. Herein we have prepared several ginkgolide derivatives carrying photoactivatable and fluorescent groups, as wells as groups where radioactive labels can be incorporated for the purpose of performing photolabeling, *ex vivo* autoradiography, and positron emission tomography (PET) studies. The first examination of the binding of native terpene trilactones and their derivatives to the cloned PAF receptor is described. These studies have shown that ginkgolide derivatives with aromatic photoactivatable substituents are potent PAF receptor antagonists with K_i values of 0.09-0.79 μM and hence excellent ligands for clarifying the binding of ginkgolides to PAF receptor by photolabeling studies. Ginkgolide derivatives incorporating both fluorescent and photoactivatable groups still retained binding affinity to the PAF receptor, and are promising ligands for photolabeling and sequencing. Finally, among the candidates for incorporation of radiotracers one compound was a potent antagonist of PAF receptor with a K_i value of 0.99 μM and is therefore a potential ligand for probing ginkgolide-PAF receptor interactions in the brain, as well as elucidating new targets for ginkgolides.

Description of the Figures

Figure 1. Terpene trilactones isolated from *Ginkgo biloba*. GA, GB, and GC are found in the leaves and root bark of *G. biloba*, but GJ is found only in the leaves, and GM only in the root bark

Figure 2. Structures of platelet-activating factor (PAF), the endogenous ligand for the PAFR, and WEB 2086, a potent and selective antagonist, both of which have been used in radioligand binding studies.

Figure 3. Derivatives **8a-c** and GC derivatives **9a-c**.

Figure 4. 10-*O*-benzophenone-7-*O*-dansyl GC (**10a**) and 10-*O*-benzophenone-1-*O*-dansyl GC (**10b**).

Figure 5. 10-benzophenonecarbonyl GC (**11**).

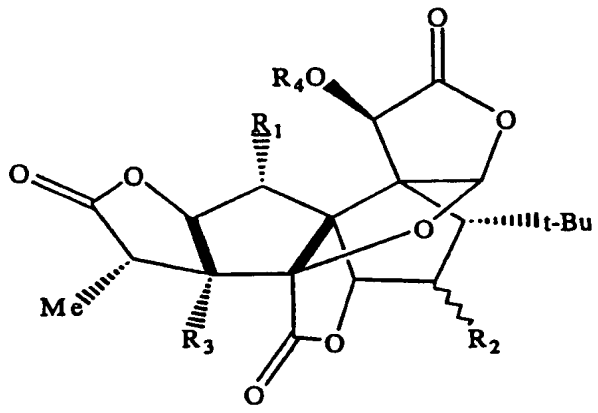
Figure 6. 7-fluoro analog of GB (**13**), 10-*O*-methyl GB (**14**), and 10-*O*-(2-fluoroethyl) GB (**15**).

Figure 7. [^{3}H]-Ginkgolide B ([^{3}H]-GB).

Figure 8. Summary of structure-activity relationship (SAR) studies of ginkgolides as PAFR antagonists. GB (**1**) is ca. 25-fold more potent than GC (**2**).

Detailed Description of the Invention

The invention provides a compound having the structure:



(I)

wherein R_1 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

wherein R_2 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

wherein R_3 is H or OH;

wherein R_4 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; and

wherein at least one of R_1, R_2, R_3, or R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety,

or an optically pure enantiomer of the compound.

In the compound, R_1 may be a fluorescent moiety and each of R_2 and R_4 may be H or OH; R_2 may be a fluorescent moiety or a radioactive moiety and each of R_1 and R_4 may be H or OH; or R_4 may be a photoactivatable moiety or a radioactive moiety and each of R_1 and R_2 may be H or OH.

The photoactivatable moiety may be a phenylazide, a purine or pyrimidine azides, a diazoacetate, a diazoketone, a nitrobenzene, or an aryldiazonium salt. In some embodiments,

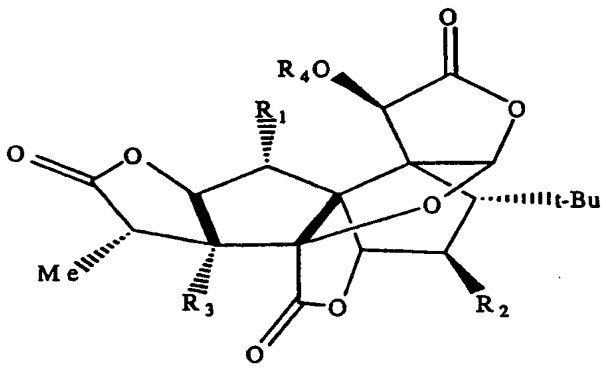
the photoactivatable moiety may be benzophenone, trifluoromethyldiazirine tetrafluorophenyl, 8-azidoadenosine, 2-azidoadenosine, or 3H,3-aryldiazirine.

The fluorescent moiety may be a fluorescent amine. In some embodiments, the fluorescent moiety may be 5-(dimemethylamino)naphthalene-sulfonyl chloride, 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, or 4-(9-Anthroyloxy)phenacyl bromide.

The radioactive moiety may be ^{11}C, ^{13}N, ^{15}O, ^{3}H or ^{18}F.

In specific embodiments of the compounds of this invention, the photoactivatable moiety may be benzophenone, trifluoromethyldiazirine or tetrafluorophenyl; the fluorescent moiety may be 5-(dimemethylamino)naphthalene-sulfonyl ("dansyl"); and the radioactive moiety may be ^{18}F, ^{11}C or ^{3}H.

In a specific embodiment the invention also provides compounds having the structure:

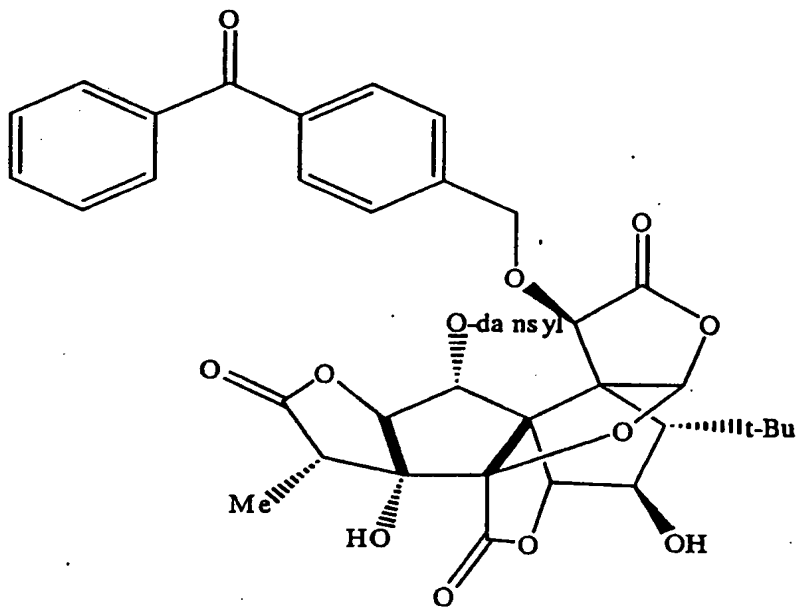


where R_1, R_2, R_3 and R_4 are defined as above.

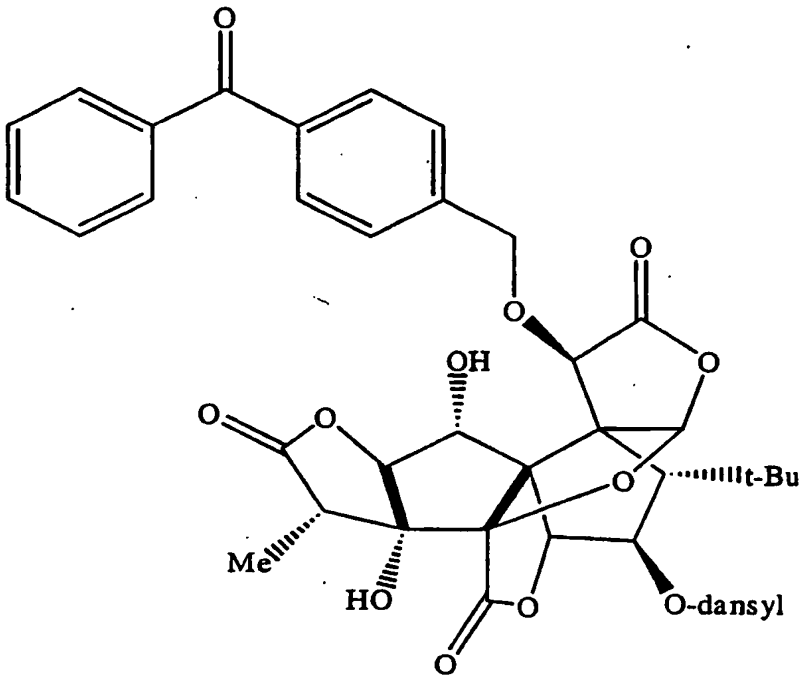
In specific embodiments, R_1 may be H, OH, a fluorescent moiety; R_2 may be H, OH, a fluorescent moiety, or a radioactive moiety; R_3 may be H or OH; and R_4 may be H, OH, a photoactivatable moiety, or a radioactive moiety.

In yet further embodiments, R_1 may be -O-dansyl; or R_2 may be -O-dansyl; or R_2 may be $-^{11}CH_3$; or R_2 may be $-CH_2CH_2{}^{18}F$; or R_2 may be ^{18}F; or R_2 may be ^{3}H; or R_4 may be a benzophenone moiety; or R_4 may be a trifluoromethyldiazirine moiety; or R_4 may be a tetrafluorophenyl azide moiety; or R_4 may be $-^{11}CH_3$; or R_4 may be $-CH_2CH_2{}^{18}F$.

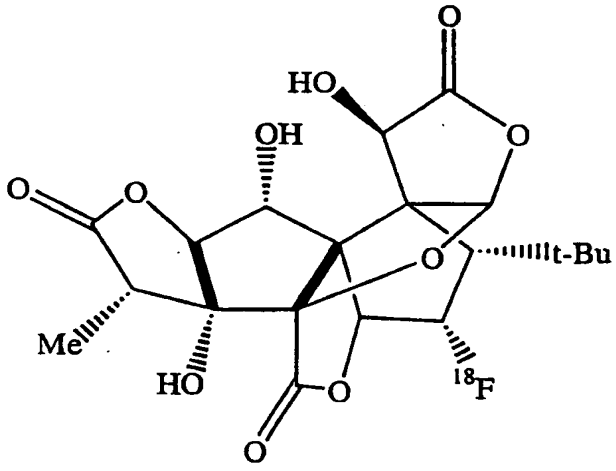
In one specific embodiment, the compound has the structure:



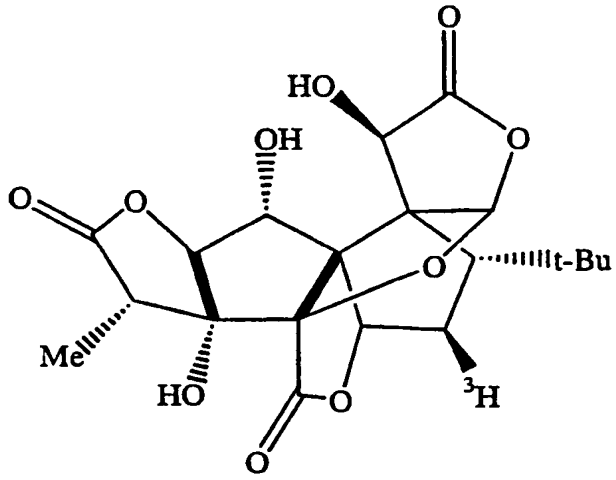
In another specific embodiment, the compound has the structure:



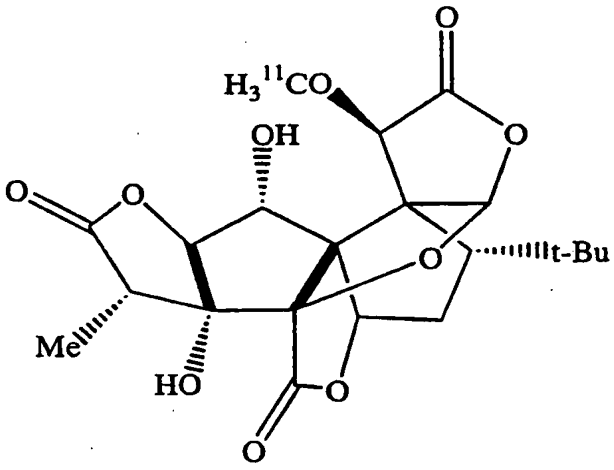
In another specific embodiment, the compound has the structure:



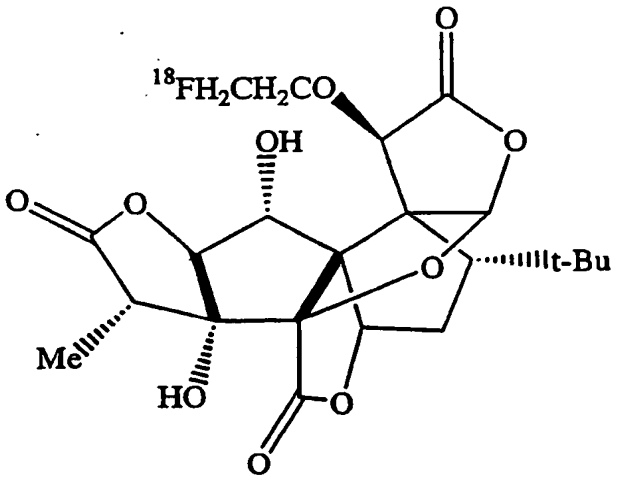
In another specific embodiment, the compound has the structure:



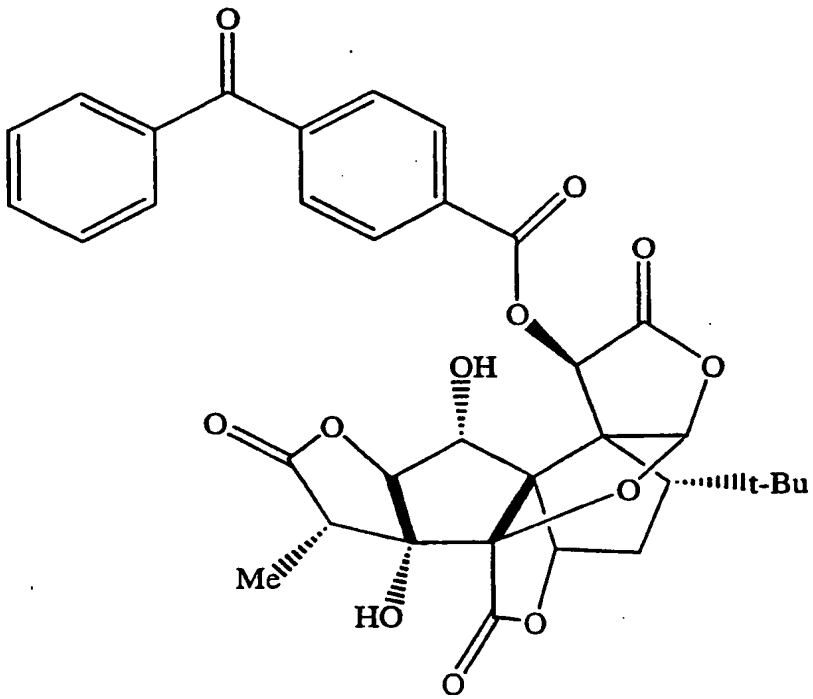
In another specific embodiment, the compound has the structure:



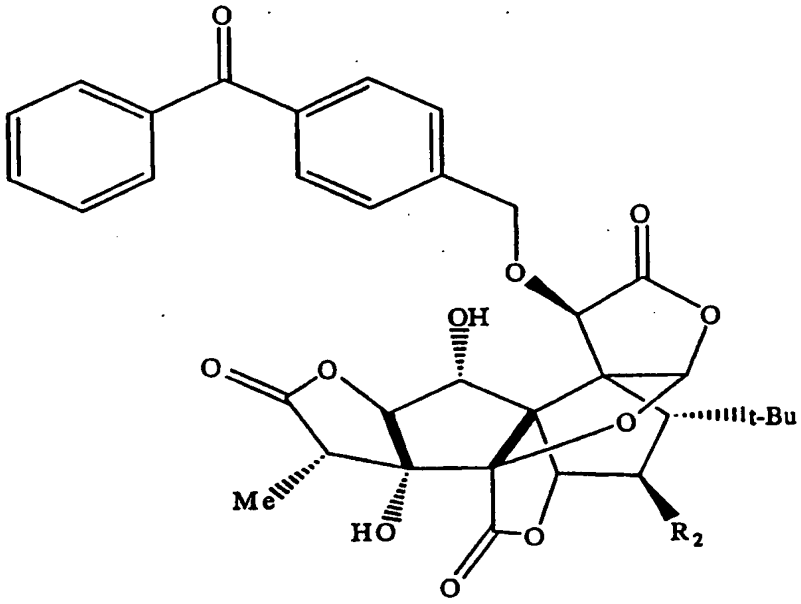
In another specific embodiment, the compound has the structure:



In another specific embodiment, the compound has the structure:

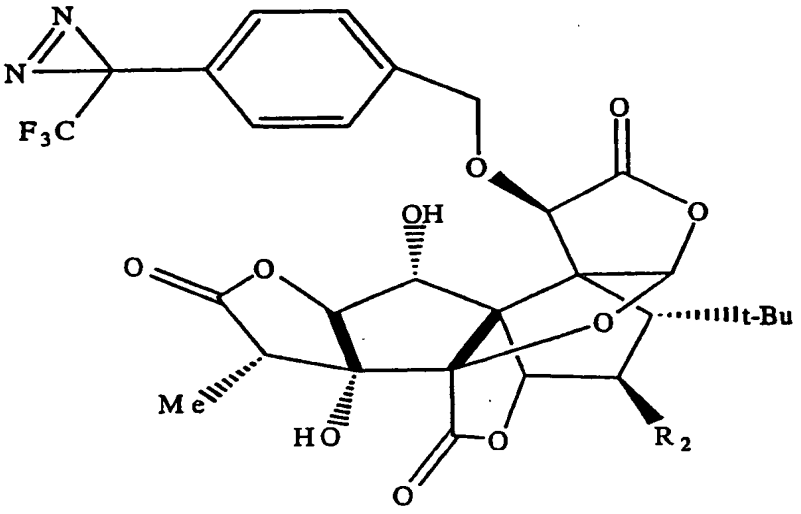


In another specific embodiment, the compound has the structure:



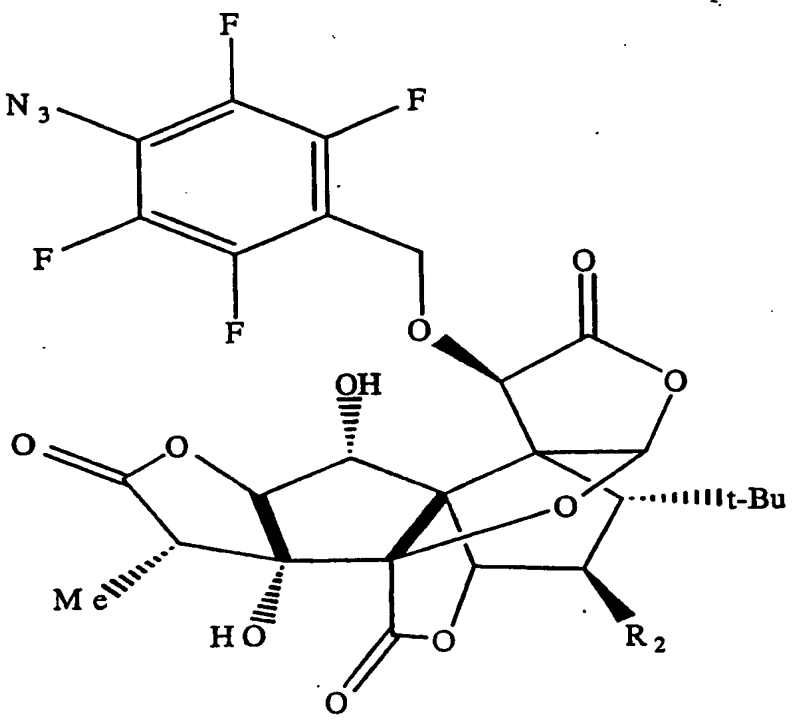
wherein R_2 is H or OH.

In another specific embodiment, the compound has the structure:



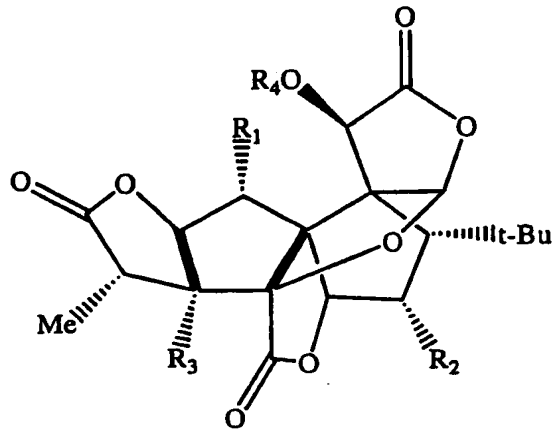
wherein R_2 is H or OH.

In another specific embodiment, the compound has the structure:



wherein R_2 is H or OH.

The invention also provides a compound having the structure:



wherein R_1 is H or OH,

wherein R_2 is H, OH, halogen, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, $-N_3$, $-COR_5$, $-CONR_5R_6$, $-CO_2R_5$, $-OCOR_5$, $-NH(OH)$, $-NR_5R_6$, $-NHCOR_5$, $-N(OH)COR_5$, $-CH_2OR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, or $-NR_5SO_2R_6$,

where R_5 and R_6 are each, independently, hydrogen, substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms;

wherein R_3 is H or OH; and

wherein R_4 is H, OH, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_6, or $-R_6$,

where A is (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8)alkynyl, which is unsubstituted or substituted by a straight or branched alkyl chain group having 1 to 5 carbon atoms;

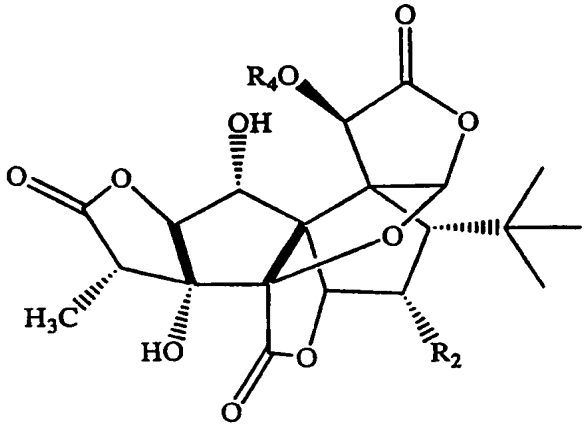
Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, substituted or unsubstituted (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen;

or an optically pure enantiomer, or a tautomer, or a salt of the compound.

In one embodiment, the compound has the structure:



wherein R_2 is Cl, F, OH, a substituted or unsubstituted, straight or branched (C_1-C_5) alkyl, (C_2-C_5) alkenyl, (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, $-N_3$, $-COR_5$, $-CONR_5R_6$, $-CO_2R_5$, $-OCOR_5$, $-NH(OH)$, $-NR_5R_6$, $-NHCOR_5$, $-N(OH)COR_5$, $-CH_2OR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, or $-NR_5SO_2R_6$,

where R_5 and R_6 are each, independently, hydrogen, substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms;

wherein R_4 is H or R_8;
wherein R_4 is R_8 when R_2 is F;

wherein R_8 is (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_6,

where A is an unsubstituted, straight chain (C_1-C_5)alkyl, (C_2-C_5) alkenyl, (C_2-C_5) alkynyl;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, hydroxy, substituted or unsubstituted (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, phenyl, phenoxy, aralkyl, or aralkyloxy, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, or $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, -CN, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a tautomer, or a salt

of the compound.

In another embodiment, R_4 is H or -A-Ar.

In another embodiment, R_4 is H or $-CH_2C_6H_5$.

In another embodiment, R_2 is OH, Cl, $-N_3$, $-OCOR_3$, or $-NR_3R_4$.

In another embodiment, R_2 is OH, Cl, $-OCOCH_3$, $-OCOCH_2C_6H_5$, $-N_3$, $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$.

In another embodiment, R_2 is OH, Cl, $-OCOCH_3$, $-OCOCH_2C_6H_5$, $-N_3$, $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$; and wherein R_4 is H or $-CH_2C_6H_5$.

In another embodiment, R_1 is OH, R_2 is F, R_3 is OH, and R_4 is H.

In another embodiment, R_2 is Cl, and R_4 is H.

In another embodiment, R_2 is $-N_3$, and R_4 is H.

In another embodiment, R_2 is $-NHCH_3$, and R_4 is H.

In another embodiment, R_2 is $-NHCH_2CH_3$, and R_4 is H.

In another embodiment, R_2 is $-OCOCH_2C_6H_5$, and R_4 is H.

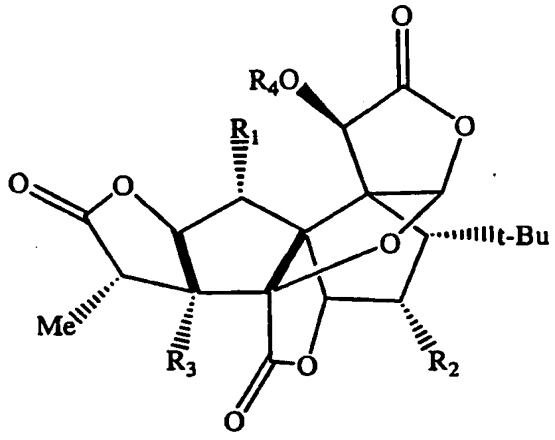
In another embodiment, R_2 is F, and R_4 is $-CH_2C_6H_5$.

In another embodiment, R_2 is Cl, and R_4 is $-CH_2C_6H_5$.

In another embodiment, R_2 is H, and R_4 is $-CH_2C_6H_5$.

In another embodiment, R_2 is OH, and wherein R_4 is $-CH_2C_6H_5$.

In another embodiment, the compound has the structure:



wherein R_1 is H or OH;

wherein R_2 is H, F, Br, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl;

wherein R_3 is H or OH; and

wherein R_4 is H, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_6, or $-R_6$,

where A is an alkylene group having 1 to 8 carbon atoms, which is unsubstituted or substituted by a straight or branched alkyl chain group having 1 to 5 carbon atoms;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, a (C_1-C_{10}) haloalkyl, an (C_1-C_{10}) alkoxy, an (C_2-C_{10}) alkenyloxy, an (C_2-C_{10}) alkynyloxy, a (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl

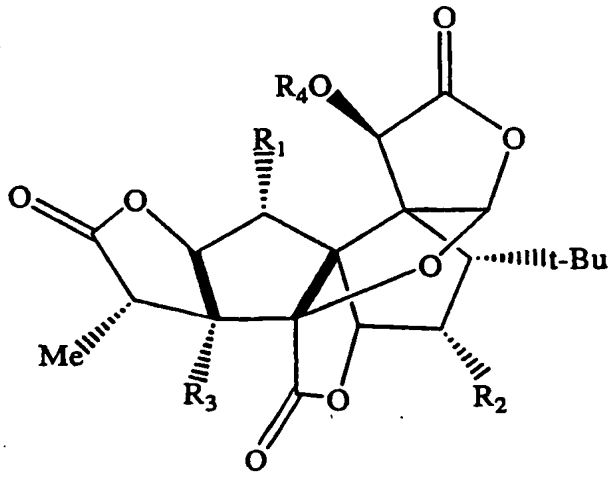
group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

In one embodiment, R_2 is F.

In another embodiment, the compound has the structure:



wherein R_1 is H, or OH;

wherein R_2 is H, OH, F, Br, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl;

wherein R_3 is H or OH; and

wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_5, or -R_6,

where A is an (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an

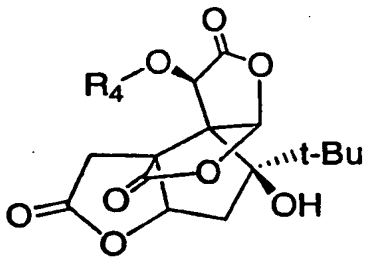
aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$, $-NHCOR_5$, $-NH(OH)$, $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, (C_1-C_{10}) alkyl or a (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, -CN, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

In another embodiment, R_2 is F.

The subject invention also provides a compound having the structure:



wherein R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety.

In one embodiment, the photoactivatable moiety is a phenylazide, a purine or pyrimidine azides, a diazoacetate, a diazoketone, a nitrobenzene, or an aryldiazonium salt.

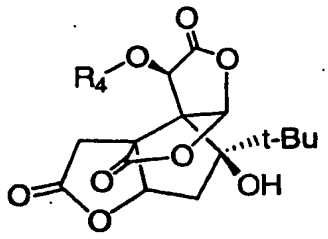
In another embodiment, the photoactivatable moiety is benzophenone, trifluoromethyldiazirine tetrafluorophenyl, 8-azidoadenosine, 2-azidoadenosine, or 3H,3-aryldiazirine.

In another embodiment, the fluorescent moiety is a fluorescent amine.

In another embodiment, the fluorescent moiety is 5-(dimemethylamino)naphthalene-sulfonyl (dansyl), 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, or 4-(9-Anthroyloxy)phenacyl bromide.

In another embodiment, the radioactive moiety is ^{18}F, ^{11}C, ^{3}H.

The invention also provides a compound having the structure:



wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_5, or $-R_6$,

where A is a (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$,

$-NHCOR_5$, $-NH(OH)$., $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, (C_1-C_{10}) alkyl or a (C_3-C_{10}) cycloalkyl group, $-SCX_3$ in which X is a halogen, -CN, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

In one embodiment of the above compounds, if any group is substituted, the substituent is halogen, hydroxyl, straight chain (C_1-C_5)alkyl, branched chain (C_3-C_5)alkyl, (C_3-C_{10})cycloalkyl, straight chain(C_1-C_5)alkylcarbonyloxy, branched chain (C_3-C_5)alkylcarbonyloxy, arylcarbonyloxy, straight chain(C_1-C_5)alkoxycarbonyloxy, branched chain(C_3-C_5)alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, straight chain(C_1-C_5)alkylcarbonyl, branched chain (C_3-C_5)alkylcarbonyl, straight chain (C_1-C_5)alkoxycarbonyl, branched chain (C_3-C_5)alkoxycarbonyl, aminocarbonyl, straight chain (C_1-C_5)alkylthiocarbonyl, branched chain (C_3-C_5)alkylthiocarbonyl, straight chain (C_1-C_5)alkoxyl, branched chain (C_1-C_5)alkoxyl, phosphate, phosphonate, cyano, amino, straight chain (C_1-C_5)alkylamino, branched chain (C_3-C_5)alkylamino, straight chain (C_1-C_5)dialkylamino, branched chain (C_3-C_5)dialkylamino, arylamino, diarylamino, straight chain (C_1-C_5)alkylarylamino, branched chain (C_3-C_5)alkylarylamino, acylamino, straight chain (C_1-C_5)alkylcarbonylamino, branched chain (C_3-C_5)alkylcarbonylamino, arylcarbonylamino, carbamoyl, ureido, amidino, imino, sulfhydryl, straight chain (C_1-C_5)alkylthio, branched chain (C_3-C_5)alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, azido, 4-10 membered heterocyclyl, straight chain (C_1-C_{30})alkylaryl, branched chain (C_3-C_{30})alkylaryl, or an aromatic or 5-6 membered heteroaromatic moiety,
which substituent may be further substituted by any of the above.

The subject invention also provides a pharmaceutically acceptable salt of above compounds, wherein the salt is the chloride, mesylate, maleate, fumarate, tartarate, hydrochloride, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate and sulfate salts.

The subject invention also provides a pharmaceutical composition

comprising a therapeutically effective amount of one of the above compounds and a pharmaceutically acceptable carrier.

The subject invention also provides a process for the manufacture of a pharmaceutical composition comprising admixing any of the above compounds with a pharmaceutically acceptable carrier.

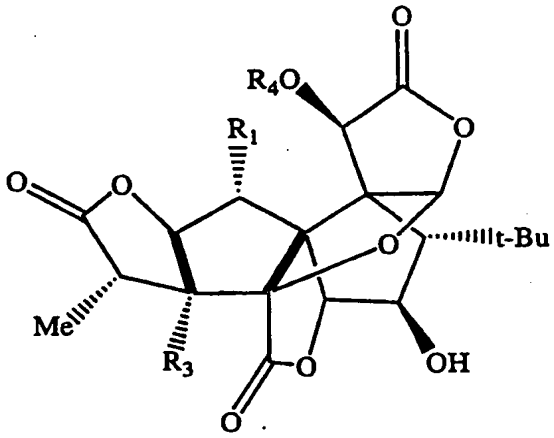
The subject invention also provides a method of inhibiting activation of the platelet-activating factor receptor (PAFR) which comprises contacting the PAFR with any of the above compounds so as to thereby inhibit activation of the PAFR.

The subject invention also provides a method of treating a platelet-activating factor (PAF) associated condition in a subject comprising administering to the subject an amount of any of the above compounds effective to treat the PAF-associated disease.

In one embodiment, the PAF-associated condition is a neurodegenerative disease, Alzheimer's disease, senile dementia, stroke, nerve cell damage due to ischemia, asthma, abnormal blood clot formation, endotoxic shock, myocardial ischemia or hyperacute rejection arising from post-renal transplant or xenoperfusion.

The subject invention also provides a process of preparing the above compound comprising the steps of:

i) reacting a compound having the structure



wherein R_1, R_3 and R_4 are as defined above,

with trifluoromethanesulfonic anhydride under inert conditions to form a triflate; and

ii) reacting the triflate of step i) with a nucleophilic reagent in a polar-aprotic solvent to substitute the triflate with the nucleophile and to form the compound.

In one embodiment of the above process, the nucleophilic reagent is sodium acetate, sodium phenylacetate, sodium azide, tetrabutylammonium fluoride hydrate, or tetrabutylammonium chloride.

In another embodiment, the polar-aprotic solvent is dichloromethane, pyridine, dimethylformamide, methylsulfoxide, or acetonitrile.

In another embodiment, the nucleophilic reagent is sodium acetate or sodium phenylacetate, further comprising hydrolyzing the product of step ii) in the presence of 1N hydrochloric acid.

In another embodiment, the nucleophilic reagent is sodium azide,

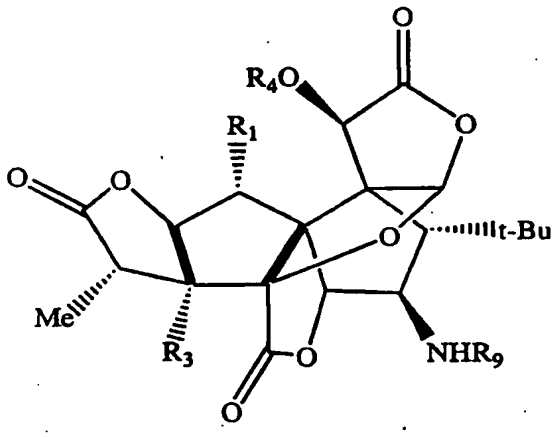
further comprising reacting the product of step ii) with hydrogen in the presence of palladium and carbon in methanol or ethanol.

In another embodiment, the nucleophile is tetrabutylammonium fluoride hydrate, further comprising reacting the product of step ii) with benzyl chloride.

The subject invention also provides a process of forming a secondary amine compound from an azide of the compound by contacting the azide of the compound with hydrogen, palladium and carbon in a polar-protic solvent.

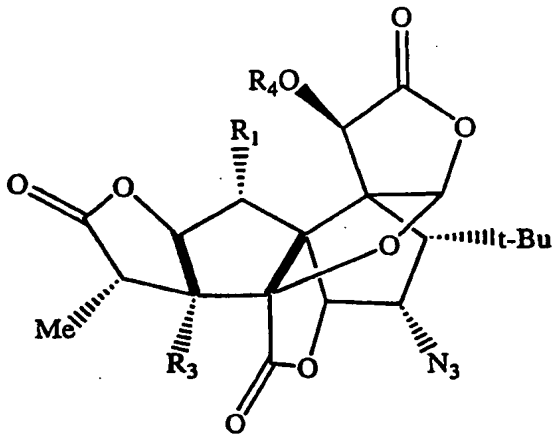
In one embodiment, the polar-protic solvent is an alcohol.

In another embodiment, the secondary amine compound has the structure:



wherein R_1, R_3 and R_4 are as defined above and R_9 is an alkyl group,

and wherein the azide of the compound has the structure:



In another embodiment, the polar-protic solvent is an alcohol.

In another embodiment, the alcohol is CH_3OH and R_9 is CH_3.

In another embodiment, the alcohol is CH_3CH_2OH and R_9 is CH_3CH_2.

The subject invention also provides a process for detecting the binding of compound (I) to a target, comprising contacting the compound with the target and detecting the binding of the compound to the target.

In one embodiment, the target is DNA.

In another embodiment, the target is a receptor.

In another embodiment, the target is an enzyme.

The subject invention also provides a process for detecting the localization of a receptor in a subject comprising administering compound (I) to the subject and detecting at any location in the subject's body to identify a point of accumulation of the compound so as to thereby localize the receptor in the subject, wherein localization of a receptor means a higher concentration of that receptor then at other points in the subject's body.

The subject invention also provides a process of identifying a target that binds compound (I), comprising contacting the compound with the target, isolating the target so as to thereby identify the target.

In one embodiment, the target is DNA.

In another embodiment, the target is a receptor.

In another embodiment, the target is an enzyme.

The subject invention also provides a process of identifying a receptor that binds compound (I) in a subject, comprising administering the compound to the subject, imaging the subject's body to identify the point of accumulation of the compound in the subject, and identifying the receptor present at the point

of accumulation of the compound, so as to thereby identify the receptor in the subject.

The invention also provides a process for detecting the binding of any of the described the compounds to a target, comprising contacting the compound with the target and detecting the binding of the compound to the target The target may be a DNA, enzyme or a receptor.

The invention also provides a process for detecting the localization of a receptor in a subject comprising administering any of the described compounds to the subject and detecting at any location in the subject's body to identify a point of accumulation of the compound so as to thereby localize the receptor in the subject, wherein localization of a receptor means a higher concentration of that receptor then at other points in the subject's body.

The invention also provides a process of identifying a target that binds any of the described compounds, comprising contacting the compound with the target and identifying target. The target may be a DNA, enzyme or a receptor.

The invention also provides a process of identifying a receptor that binds to any of the described compounds in a subject, comprising administering the compound to the subject, imaging the subject's body to identify the point of accumulation of the compound in the subject, and identifying the receptor present at the point of accumulation of the compound, thereby identifying the receptor in the subject.

The photoactivatable moieties react with a receptor, enzyme or other target upon irradiation and enable researchers to identify the targets of compounds, to determine the affinity and selectivity of the drug-target interaction, and to identify the binding site on the target. Examples are presented from three

fundamentally different approaches: (1) photoaffinity labeling of target macromolecules; (2) photoactivation and release of "caged ligands"; and (3) photoimmobilization of ligands onto surfaces. A number of photoactivatable moieties are described in the literature, for example, aryl azides, which, when photoactivated to yield aryl nitrenes, can label any binding site containing carbon-hydrogen bonds by insertion into the C--H bond (Galardy, et al., J. Biol. Chem., 249: 350 (1974); U.S. Patent No. 4,689,310; and U.S. Pat. No. 4,716,122); and a number of others are described in U.S. Patent No. 6,077,698, the contents of which are hereby incorporated by reference.

The photoactivatable groups can be used for treatment as well as screening studies and diagnostics. Photoactivatable groups can be used to irreversibly bind compounds to their targets. Thus, the subject invention also provides compounds useful in methods of treatment where a desired compound is irreversibly bound to its target.

The photoactivatable groups may be phenylazides, purine and pyrimidine azides, 8-azidoadenosine, 2-azidoadenosine, diazoacetates, diazoketones, nitrobenzenes, aryldiazonium salts, or 3H,3-aryldiazirines. The preferred photoactivatable moieties for use with ginkgolides are benzophenone, trifluoromethyldiazirine and tetrafluorophenyl.

The fluorescent moiety, for example, may be 5-(dimemethylamino)naphthalene-sulfonyl chloride (dansyl chloride), a fluorescent amine such as 1-pyrenemethylamine, or any number of other groups readily available from Molecular Probes - http://www.probes.com/, the contents of which are hereby incorporated by reference. Other specific groups which are useful in this invention are 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, 3-Bromomethyl-6,7-

dimethoxy-1-methyl-2(1H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, and 4-(9-Anthroyloxy)phenacyl bromide.

Radioactive moieties are widely known in the art and include radionuclides, radionuclides covalently attached to other groups, and metal chelates. The appropriate ginkgolide-based radioligands can be prepared using known radioactive moieties to suit the environment of use and detection method. Gamma-emitter and positron-emitter radionuclides are well-known in the art and include ^{111}In, ^{198}Au, ^{113}Ag, ^{111}Ag, ^{123}I, ^{125}I, ^{130}I, ^{131}I, ^{133}I, ^{135}I, ^{47}Sc, ^{72}As, ^{72}Se, ^{90}Y, ^{88}Y, ^{97}Ru, ^{100}Pd, ^{109}Pd, ^{105}Rh, ^{128}Ba, ^{197}Hg, ^{203}Pb, ^{212}Pb, ^{67}Ga, ^{68}Ga, ^{64}Cu, ^{67}Cr, ^{97}Ru, ^{75}Br, ^{76}Br, ^{77}Br, ^{99m}Tc, ^{11}C, ^{13}N, ^{15}O, ^{3}H and ^{18}F. For positron emission tomography (PET) studies contemplated by this disclosure, ginkgolide derivatives labeled with the radionuclides [^{18}F]- and [^{11}C] possessing half lives of 110 min and 20 min, respectively, are preferred. While [^{3}H] is preferred for other radioactivity based studies.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Methods

SYNTHESIS

Ginkgolides and bilobalide (**1-6**) were obtained as previously described (Nakanishi, 1967). The syntheses of ginkgolide derivatives are outlined in Figures 3-7, and details appear below:

General procedures.

Anhydrous solvents were dried by eluting through alumina columns. Triethylamine was freshly distilled from NaOH pellets. Unless otherwise noted, materials were obtained from a commercial supplier and were used without further purification. All reactions were performed in predried glassware under argon or nitrogen, and all reactions of involving azides or diazirines were performed in dim red light. Flash column chromatography was performed using ICN silica gel (32-63 mesh) or ICN silica gel (32-63 mesh) impregnated with sodium acetate. [van Beek, T. A. & Lelyved, G. P. (1993) *Phytochem. Anal.* **4**, 109-114].

Thin-layer chromatography was carried out using pre-coated silica gel 60 F_{254} plates with thickness of 0.25 μm. Spots were observed at 254 nm, and by staining with acetic anhydride, or cerium/molybdenum in H_2SO_4. ^1H and ^{13}C NMR spectra were obtained on Bruker DMX 300 or 400 MHZ spectrometers and are reported in parts per million (ppm) relative to internal solvent signal, with coupling constants (J) in Hertz (Hz). For ^{19}F NMR spectra hexafluorobenzene (-162.9 ppm) was used as internal standard. High resolution mass spectra (HRMS) were measured on a JEOL JMS-HX110/100A HF mass spectrometers using a 3-nitrobenzyl alcohol (NBA) matrix and Xe ionizing gas.

Example 1 (Figure 3)

Synthesis of 8a-c and 9a-c - General synthetic procedure. GB (2) or GC (3) (0.07 mmol) was dissolved in THF (4 mL) and KH (0.008 g, 0.24 mmol) was added at room temperature. The reaction mixture was stirred for 10 min., when a solution of **7a**, **7b**, or **7c** (0.212 mmol) in THF (1 mL) was added dropwise. The reaction was stirred at room temperature for 4 hours. The solution was then cooled to 0 °C and concentrated HCl (0.3 mL) was added. The mixture was diluted with H_2O (10 mL), extracted with EtOAc (3 x 10 mL) and washed with sat. aq. NH_4Cl-solution (30 mL), brine (30mL) and water (30 mL). The organic phase was dried ($MgSO_4$) and removed *in vacuo*.

Purification. The crude material is purified by flash column chromatography using either A: $CHCl_3$/MeOH (100:1 and 50:1), B: $CHCl_3$/MeOH (30:1 and 20:1), or C: cyclohexane/acetone (3:1 and 2:1) giving a white solid.

10-*O*-benzophenone ginkgolide B (8a). Purified by method B. Yield: 0.035 g (78%). ^{1}H NMR (400 MHZ, CD_3OD): δ 1.13 (s, *tert*-butyl), 1.24 (d, J = 7.1, CH_3), 1.92 (dd, J = 14.3, 4.5, 8-H), 2.07 (td, J = 13.9, 4.4, 7α-H), 2.27 (dd, J = 13.5, 4.6, 7β-H), 3.06 (q, J = 7.1, 14-H), 4.31 (d, J = 7.2, 1-H), 4.55 (d, J = 7.2, 2-H), 4.85 (d, J = 11.5, benzylic-H, 1H), 5.28 (s, 10-H), 5.42 (d, J = 4.0, 6-H), 5.59 (d, J = 11.5, benzylic-H, 1H), 6.15 (s, 12-H), 7.53-7.60 (m, Ar-H, 4H), 7.65-7.67 (m, Ar-H, 1H), 7.77-7.82 (m, Ar-H, 4H). ^{13}C NMR (100 MHZ, CD_3OD): δ 7.25, 28.46 (3C), 32.18, 37.26, 42.29, 49.61, 68.21, 72.59, 72.80, 74.45, 76.76, 79.48, 83.53, 93.15, 99.78, 110.83, 127.96 (2C), 128.58 (2C), 130.03 (2C), 130.52 (2C), 132.94, 137.76 (2C), 141.67, 171.52, 172.70, 177.33, 196.45. HRMS: $C_{34}H_{34}O_{11}$ requires M + Na at *m/z* 641.1999, found 641.2018.

10-*O*-(trifluoromethyl-3H-diazirine)benzyl ginkgolide B (8b). Purified by method B. Yield: 0.024g (59%). ^{1}H NMR (400 MHz,

CD_3OD): δ 1.11 (s, *tert*-butyl), 1.23 (d, J = 7.1, CH_3), 1.89 (dd, J = 14.3, 4.3, 8-H), 2.01 (td, J = 13.9, 4.3, 7α-H), 2.25 (dd, J = 13.4, 4.4, 7β-H), 3.05 (q, J = 7.1, 14-H), 4.27 (d, J = 7.3, 1-H), 4.53 (d, J = 7.3, 2-H), 4.77 (d, J = 11.2, benzylic-H, 1H), 5.24 (s, 10-H), 5.39 (d, J = 3.9, 6-H), 5.51 (d, J = 11.2, benzylic-H, 1H), 6.14 (s, 12-H), 7.29 and 7.53 (AA'BB' system, Ar-H, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.67, 21.57 (q, $^2J_{CF}$ = 40.9, CCF_3), 29.56 (3C), 32.65, 37.49, 49.31, 68.07, 72.88, 73.57, 74.57, 76.56, 77.65, 80.08, 83.90, 90.90, 99.05, 110.68, 122.33 (q, $^1J_{CF}$ = 274.3, CF_3), 127.83 (2C), 129.53 (2C), 131.06, 136.44, 171.25, 171.50, 175.87. ^{19}F NMR (282 MHz, $CDCl_3$): δ -66.23 (s, 3F). HRMS: $C_{29}H_{29}F_3N_2O_{10}$ requires M + 1 at *m/z* 623.1853, found 623.1834.

10-*O*-tetrafluorobenzylazide ginkgolide B (8c). Purified by method B.Yield:0.023 g (50%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, *tert*-butyl), 1.32 (d, J = 7.0, CH_3), 1.84-1.97 (m, 8-H and 7α-H), 2.27-2.33 (m, 7β-H), 2.84 (d, J = 3.5, 1-OH), 2.99 (s, 3-OH), 3.06 (q, J = 7.0, 14-H), 4.29 (dd, J = 7.9, 3.5, 1-H), 4.61 (d, J = 7.9, 2-H), 4.81 (d, J = 10.7, benzylic-H, 1H), 4.94 (s, 10-H), 5.39 (d, J = 3.4, 6-H), 5.64 (d, J = 10.7, benzylic-H, 1H), 6.03 (s, 12-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.70, 29.52 (3C), 32.62, 37.37, 42.03, 49.30, 61.21, 68.11, 72.79, 74.65, 80.07, 83.89, 91.00, 99.12, 108.95, 110.73, 139.71, 142.24, 144.45, 147.10, 170.69, 171.45, 175.83. ^{19}F NMR (282 MHz, $CDCl_3$): δ -143.31 (m, 2F), -150.85 (m, 2F). HRMS: $C_{27}H_{25}F_4N_3O_{10}$ requires M + 1 at *m/z* 628.1554, found 628.1565.

10-*O*-benzophenone ginkgolide C (9a). Purified by method A. Yield: 0.023g (64%). ^1H NMR (400, MHz, CD_3OD): δ 1.20 (s, *tert*-butyl), 1.24 (d, J = 7.1, CH_3,), 1.78 (d, J = 12.5, 8-H), 3.04 (q, J = 7.1, 14-H), 4.21 (dd, J = 12.5, 4.3, 7-H), 4.28 (d, J = 7.0, 1-H), 4.54 (d, J = 7.0, 2-H), 4.87 (d, J = 11.6, benzylic-H, 1H), 5.13 (d, J = 4.3, 6-H), 5.28 (s, 10-H), 5.60 (d, J = 11.6, benzylic-H, 1H), 6.17 (s, 12-H) 7.53-7.61 (m, Ar-

H, 4H), 7.65-7.67 (m, Ar-H, 1H), 7.77-7.83 (m, Ar-H, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.34, 28.50 (3C), 32.12, 42.26, 50.00, 64.48, 67.40, 72.77, 74.28, 75.14, 76.74, 79.49, 83.55, 93.28, 99.54, 110.63, 127.95 (2C), 128.59 (2C), 130.03 (2C), 130.53 (2C), 132.96, 137.68 (2C), 141.65, 171.41, 172.55, 177.27, 197.03. HRMS: $C_{34}H_{34}O_{12}$ requires M + 1 at *m/z* 635.2129, found 635.2098.

10-*O*-(trifluoromethyl-3H-diazirine)benzyl ginkgolide C (9b). Purified by method A.Yield: 0.023 g (51%). ^1H NMR (400 MHz, CD_3OD): δ 1.17 (s, *tert*-butyl), 1.24 (d, *J* = 7.1, CH_3,), 1.76 (d, *J* = 12.5, 8-H), 3.02 (q, *J* = 7.1, 14-H), 4.15 (dd, *J* = 12.5, 4.3, 7-H) 4.24 (d, *J* = 7.0, 1-H), 4.52 (d, *J* = 7.0, 2-H), 4.79 (d, *J* = 11.3, benzylic-H, 1H), 5.10 (d, *J* = 4.3, 6-H), 5.23 (s, 10-H), 5.52 (d, *J* = 11.3, benzylic-H, 1H), 6.15 (s, 12-H), 7.29 and 7.54 (AA'BB' system, aromatic-H, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.65, 23.77 (q, $^2J_{CF}$ = 38.9, CCF_3), 29.52 (3C), 32.65, 41.94, 50.92, 64.43, 67.48, 73.89, 74.30, 76.03, 76.34, 79.63, 83.90, 90.91, 98.94, 110.53, 122.32 (q, $^1J_{CF}$ = 275.0, CF_3), 127.94 (2C), 129.68 (2C), 131.26, 136.07, 170.97, 171.07, 175.69. ^{19}F NMR (282 MHz, $CDCl_3$): δ -66.23 (s, 3F). HRMS: $C_{29}H_{29}F_3N_2O_{11}$ requires M + 1 at *m/z* 639.1802, found 639.1790.

10-*O*-tetrafluorobenzylazide ginkgolide C (9c). Purified by method C. Yield: 0.080g (54%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.22 (s, *tert*-butyl), 1.33 (d, *J* = 7.0, CH_3,), 1.71 (d, *J* = 12.4, 8-H), 2.33 (d, *J* = 10.6, 7-OH), 2.88 (d, *J* = 3.4, 1-OH), 3.01 (s, 3-OH), 3.08 (q, *J* = 7.0, 14-H), 4.08 (m, 7-H) 4.27 (dd, *J* = 7.8, 3.4, 1-H), 4.62 (d, *J* = 7.8, 2-H), 4.83 (d, *J* = 10.7, benzylic-H, 1H), 4.96 (s, 10-H), 5.09 (d, *J* = 4.4, 6-H), 5.58 (d, *J* = 10.7, benzylic-H, 1H), 6.04 (s, 12-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.64, 29.42 (3C), 32.59, 42.08, 50.64, 51.16, 61.47, 64.35, 67.32, 74.27, 75.88, 79.64, 83.88, 91.26, 99.14, 110.71, 120-150 (m, 6C), 170.72, 171.17, 176.29. ^{19}F NMR (282 MHz, $CDCl_3$): δ -143.56 (m, 2F), -151.08 (m, 2F); HRMS: $C_{27}H_{25}F_4N_3O_{11}$ requires M +

1 at *m/z* 644.1503, found 644.1527.

Example 2A (Figure 4)

10-*O*-benzophenone-7-*O*-dansyl ginkgolide C (10a). A solution of dansyl chloride (0.010 g, 0.035 mmol) in acetonitrile (0.3 mL) was added to a solution of **9a** (0.020 g, 0.032 mmol) and DMAP (0.008 g, 0.063 mmol) in acetonitrile (1.5 mL). The reaction mixture was stirred for 16 h at room temperature, then a sat. aq. NH_4Cl0-solution (2 mL) was added, and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic phases were washed with sat. aq. NaCl-solution (3 x 15 mL), dried ($MgSO_4$) and removed *in vacuo*. The crude product was purified by flash column chromatography eluting with cyclohexane/acetone (2:1) to give the product as a slightly yellow solid (0.015 g, 56%). ^1H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, *tert*-butyl), 1.09 (d, J = 7.2, CH_3,), 1.94 (d, J = 12.5, 8-H), 2.81 [m, 14-H and $N(CH_3)_2$], 4.26 (t, J = 5.3, 1-H), 4.53 (d, J = 5.4, 2-H), 4.79 (d, J = 13.2, benzylic-H, 1H), 4.89 (dd, J = 12.5, 4.0, 7-H), 5.19 (d, J = 4.0, 6-H), 5.23 (s, 10-H), 5.46 (d, J = 13.2, benzylic-H, 1H), 6.07 (d, J = 5.3, 1-OH), 6.21 (s, 12-H), 6.51 (s, 3-OH), 7.28-7.30 (m, Ar-H, 1H), 7.50-7.70 (m, Ar-H, 7H), 7.79-7.82 (m, Ar-H, 4H), 8.18-8.20 (m, Ar-H, 2H), 8.54-8.56 (m, Ar-H, 1H);). HRMS: $C_{46}H_{45}NO_{14}S$ requires M + 1 at *m/z* 868.2639, found 868.2642.

Example 2B

10-*O*-benzophenone-1-*O*-dansyl ginkgolide C (10b). Synthesized as **10a**, but using 2 equivalents of dansyl chloride (instead of 1.1 equivalent) give rise to a 1:1 mixture of **10a** and **10b**. The two products were separated on analytical TLC giving **10b** (0.008 g, 30%) as a slightly yellow solid. ^1H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, *tert*-butyl), 1.16 (d, J = 7.6, CH_3,), 1.78 (d, J = 12.5, 8-H), 2.80 [s, $N(CH_3)_2$], 2.97 (q, J = 7.6, 14-H), 4.22 (d, J = 3.8, 1-H), 4.26 (m, 7-H), 4.57 (d, J = 3.9, 6-H), 4.80 (d, J = 13.2, benzylic-H, 1H), 5.20 (d, J = 3.8, 2-H), 5.30 (s, 10-

H), 5.31 (d, J = 4.9, 7-OH), 5.49 (d, J = 13.2, benzylic-H, 1H), 5.95 (s, 12-H), 5.98 (s, 3-OH), 7.25-7.27 (m, Ar-H, 1H), 7.54-7.79 (m, Ar-H, 11H), 8.18-8.24 (m, Ar-H, 2H), 8.47-8.49 (m, Ar-H, 1H);). HRMS: $C_{46}H_{45}NO_{14}S$ requires M + 1 at m/z 868.2639, found 868.2668.

Example 3 (Figure 5)

10-*O*-benzoylbenzoic ginkgolide C (11). 4-Benzoylbenzoic acid (0.018 g, 0.08 mmol) and **2** (0.028 g, 0.07 mmol) was dissolved in THF (5 mL), and the mixture cooled to 0 °C. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide HCl (EDC) (0.018 g, 0.092 mmol) and DMAP (0.002 g, 0.01 mmol) was added, and the reaction mixture stirred at 0 °C for 1 h, and continued overnight at room temperature. The solvent was removed *in vacuo*, the crude product dissolved in EtOAc (20 mL), and washed with a sat. 5% $NaHCO_3$-solution (20 mL) and brine (20 mL). The organic fraction was dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The crude product was purified by flash column chromatography eluting with hexane/EtOAc (2:1) to give the product as white crystals (0.026 g, 62%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.07 (s, *tert*-butyl), 1.26 (d, J = 7.1, CH_3), 1.98-2.10 (m, 8-H and 7α-H), 2.30-2.36 (m, 7β-H), 3.12 (q, J = 7.1, 14-H), 4.37 (d, J = 6.5, 1-H), 4.55 (d, J = 6.5, 2-H), 5.66 (d, J = 3.2, 6-H), 6.32 (s, 10-H), 6.45 (s, 12-H), 7.54-7.58 (m, Ar-H, 2H), 7.67-7.69 (m, Ar-H, 1H), 7.80-7.83 (m, Ar-H, 2H), 7.86-7.88 (m, Ar-H, 2H), 8.42-8.44 (m, Ar-H, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.42, 28.22 (3C), 32.16, 37.27, 42.29, 49.42, 67.81, 70.64, 72.74, 74.42, 79.29, 83.64, 95.13, 100.51, 111.12, 128.73 (2C), 129.92 (2C), 130.17 (2C), 130.58 (2C), 131.61, 133.41, 137.06, 142.66, 164.56, 168.93, 171.41, 177.33, 196.48. HRMS: $C_{34}H_{31}O_{12}$ requires M + Na at m/z 655.1791, found 655.1790.

Example 4A (Figure 6)

7-trifluoromethanesulfonyloxy ginkgolide B (12). Trifluoromethanesulfonic anhydride (0.2168 mL, 1.281 mmol) was

added dropwise to a cooled solution of CH_2Cl_2 (2.84 mL) and pyridine (0.115 mL, 1.405 mmol) at - 20 °C. The solution was added dropwise to a solution of **3** (0.500 g, 1.134 mmol) in pyridine (4.83 mL) and the reaction was stirred for 2 hours at -20°. The reaction mixture was heated to room temperature and the solvent was *in vacuo*. The residue was dissolved in EtOAc (30 mL) and washed with 1M HCl (30 mL), and an aqueous, saturated NaCl solution (30, mL). The organic phase was dried ($MgSO_4$), then treated with activated carbon and filtered through Celite. The solvent was removed *in vacuo* and remaining solid was precipitated from heptane/methyl *tert*-butyl ether (2:1) to give a colorless solid. The solid was purified by flash column chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1, 20:1:1, 10:1:1) give the product as white crystals (0.61 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (s, *tert*-butyl), 1.13 (d, J = 9.6 Hz, CH_3,), 2.22 (d, J = 16.5, 8-H), 2.82 (q, J = 9.6 Hz, 14-H), 4.15 (dd, J = 8.0, 5.9 Hz, 1-H), 4.73 (d, J = 8.0 Hz, 2-H), 5.08 (d, J = 7.4 Hz, 10-H), 5.24 (dd, J = 16.5, 5.6 Hz, 7-H) 5.41 (d, J = 5.6 Hz, 6-H), 5.54 (d, J = 5.9 Hz, 1-OH), 6.20 (s, 12-H), 6.62 (s, 3-OH), 7.63 (d, J = 7.4 Hz, 10-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.1, 29.2 (3C), 32.6, 42.1, 49.0, 64.2, 68.1, 69.4, 74.4, 75.2, 84.0, 86.3, 93.2, 99.9, 109.4, 118.6 (q, $^1J_{CF}$ = 316.6, CF_3), 173.9, 176.9, 179.2. HRMS: $C_{21}H_{23}F_3O_{13}S$ requires M + 1 at m/z 573.0890, found 573.0872.

Example 4B

7-Fluoro ginkgolide B (13). 7-trifluoromethanesulfonyloxy-ginkgolide B (**12**) (0.035 g, 0.061 mmol) and and tetrabutylammonium fluoride hydrate (0.038 g, 0.145 mmol) were dissolved in acetonitrile (0.2 mL) and heated at 80°C for 30 min. The crude product was applied on an HPLC semi-preparative C18 column (7.8mm x 30cmL) with a mobile phase of MeOH/$H_2$0 (40:60) at a flow of 2mL/min. The HPLC purified product was purified by flash column chromatography eluting with $CHCl_3$/MeOH (25:1) to give the final product as a white solid (0.016 g, 60%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.25 (s, *tert*-butyl), 1.26 (d,

J = 7.1, CH_3), 1.94 (dd, J_{HF} =45.5, J = 2.3, 8-H), 3.05 (q, J = 7.1, 14-H), 4.24 (d, J = 8.0, 1-H), 4.59 (d, J = 8.0, 2-H), 5.18 (s, 10-H), 5.35 (d, J_{HF} = 10.9, 6 -H), 5.38 (dd, J_{HF} = 48.8, J = 2.3, 7-H), 6.14 (s, 12-H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.0, 29.5 (3 C), 32.9, 42.4, 53.7 ($^2J_{CF}$ = 20.4), 68.8, 69.1, 71.5, 74.5, 79.5 ($^2J_{CF}$ = 36.0), 83.7, 91.7, 96.9 ($^1J_{CF}$ = 184.2), 98.8, 111.6, 171.2, 173.9, 177.1. HRMS: $C_{20}H_{23}FO_{10}$ requires M + 1 at m/z 443.1354, found 443.1370.

Example 4C

10-*O*-Methyl ginkgolide B (14). To a suspension of ginkgolide B (0.030 g, 0.071 mmol) and K_2CO_3 (0.030 g, 0.212 mmol) in acetonitrile (0.5 mL) was added iodomethane (0.020 g, 0.141 mmol). The reaction mixture was heated under reflux for 30 min. This reaction resulted in a mixture of 10-methoxy-ginkgolide B and 1-methoxy ginkgolide B in a ratio of 4 to 1. 10-Methoxy-ginkgolide B was separated using an HPLC semi-preparative C18 column (7.8mm x 30cmL) with a mobile phase of MeOH/H_2O (40:60) at a flow of 2mL/min to give the final product as a white solid (0.017 g, 56%). ^1H NMR (400 MHz, CD_3OD): δ 1.14 (s, *tert*-butyl), 1.24 (d, J = 7.1, CH_3), 1.90 (dd, J = 14.2, 4.6, 8-H), 2.06 (td, J = 13.9, 4.3, 7α-H), 2.25 (dd, J = 13.6, 4.6, 7β-H), 3.05 (q, J = 7.1, 14-H), 3.80 (s, OCH_3), 4.24 (d, J = 7.4, 1-H), 4.57 (d, J = 7.4, 2-H), 4.93 (s, 10-H), 5.43 (d, J = 4.1, 6-H), 6.10 (s, 12-H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.6, 7.0, 29.6 (3 C), 32.9, 37.6, 41.8, 48.8, 68.6, 70.5, 71.5, 74.4, 80.4, 82.2, 84.8, 90.9, 98.2, 110.3, 171.1, 173.0, 175.4. [Hu, L., Chen, Z., Xie, Y., Jiang, Y., & Zhen, H. (2000) *Bioorg. Med. Chem.* **8**, 1515-1521]

Example 4D

10-*O*-(2-Fluoroethyl) ginkgolide B (15). Ginkgolide B (2) (0.031g, 0.073 mmol) and 1-bromo-2-fluoroethane (0.102 g, 0.803 mmol) were dissolved in acetonitrile/DMF (4:1 0.25 mL) and heated at 80°C for 30 min in the presence of tetrabutylammonium hydroxide (1M in MeOH, 0.125 mL). The crude product was a

mixture of 10-fluoroethoxy ginkgolide B and 1-fluoroethoxy ginkgolide B in a 5 to 1 ratio (as determined by ^{1}H NMR). The crude product was applied on an HPLC semi-preparative C18 column (7.8mm i.d. x 30cmL) with a mobile phase of MeOH/$H_2$0 (50:50) at a flow of 2mL/min The HPLC purified product was purified by flash column chromatography eluting with $CHCl_3$/MeOH (50:1 and 25:1) to give the final product as a white solid (0.022 g, 63%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.14 (s, *tert*-butyl), 1.25 (d, J = 7.1, CH_3), 1.93 (dd, J = 14.3, 4.7, 8-H), 2.10 (td, J = 14.0, 4.3, 7α-H), 2.28 (dd, J = 13.6, 4.7, 7β-H), 3.05 (q, J = 7.1, 14-H), 3.90-4.01 (m, 1H, FCH_2CH_2O), 4.27 (d, J = 7.4, 1-H), 4.53-4.78 (m, 3H, FCH_2CH_2O), 4.60 (d, J = 7.4, 2-H), 5.15 (s, 10-H), 5.45 (d, J = 4.1, 6-H), 6.13 (s, 12-H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.1, 28.4 (3 C), 32.1, 37.1, 42.3, 49.6, 68.1, 70.4 ($^2J_{CF}$ = 21.0), 72.7, 74.5, 77.8 ($^1J_{CF}$ = 182.6), 81.7, 83.4, 83.9, 92.9, 99.6, 110.8, 171.5, 172.6, 177.3. HRMS: $C_{22}H_{27}FO_{10}$ requires M + 1 at m/z 471.1667, found 471.1687.

Example 5 (Figure 7)

Synthesis of [^{3}H]-Ginkgolide B

Initially, nBu$_4$NB^{3}H$_4$ was synthesized as follows:

NaB^3H_4 (4.69 mg, 100 μmol specific activity 100 Ci/mmol, total activity 100 mCi) and NaOH (0.20 mg, 5 μmol) is dissolved in ^3H_2O (100 μL, specific activity 5 Ci/g, total activity 500 mCi) in a vial. nBu$_4$NCl (18.53 mg, 66.7 μmol) in ^3H_2O (100 μL, specific activity 5 Ci/g, total activity 500 mCi) is added and the reaction mixture is stirred for 1 min. CH_2Cl_2 (500 μL) is added to the vial, the mixture is shaken, the water layer is removed, $MgSO_4$ is added and the suspension stirred. The CH_2Cl_2 solution is filtered through $MgSO_4$ (in a syringe) into vial, and the solvent is removed by flowing nitrogen over the solution, and heating.

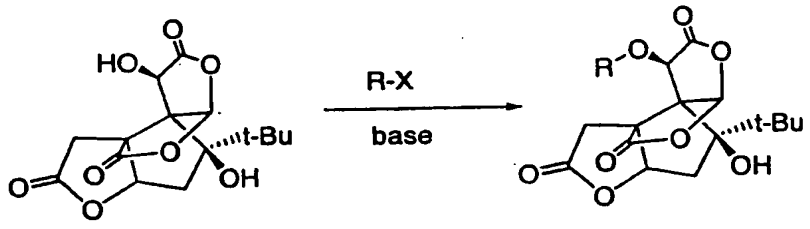
Then, the synthesis of [^{3}H]-Ginkgolide B followed (Figure 7).

nBu$_4$NB^{3}H$_4$ (1.1 mg) in dry THF (20 μL) is added to a solution of

7-triflate-Ginkgolide C (14.5 mg, in dry THF (100 μL) precooled to 0 °C. The mixture is stirred for 1.5 h at room temperature. MeOH (25 μL) is added, the mixture is shaken, and the solvent removed by flowing nitrogen over the solution, and heating. The residue is dissolved in acetonitrile (50 μL) and a mixture of H_2O/CH_3CN (1:1, 50 μL), and the solution is injected into the (preparative) HPLC (the product has a retention time of *ca.* 9.6 min.). Fractions is collected every 30 s (until 8 min.) then every 20 s, and an aliquot (5 μL) is taken into a scintillation vial, scintillation liquid is added and the vials is placed in a scintillation counter. Fractions corresponding to the peak of [^{3}H]-GB are collected and diluted with water, and passed through a C18 Sep-pak column, that is washed with water and [^{3}H]-GB is eluted with absolute ethanol into a vial, and the solvent is removed to give the product as a white solid (total activity 2.4 mCi, specific activity 3.8 mCi/μmol).

Example 6

Bilobalide (6) derivatives. Bilobalide derivatives having modifications at the 10-OH corresponding position are prepared following the procedures used herein to prepare Ginkgolide derivatives having modifications at the 10-OH position. Derivatization of Bilobalide (BB, 6) is performed as follows:



Bilobalide (BB, 6)

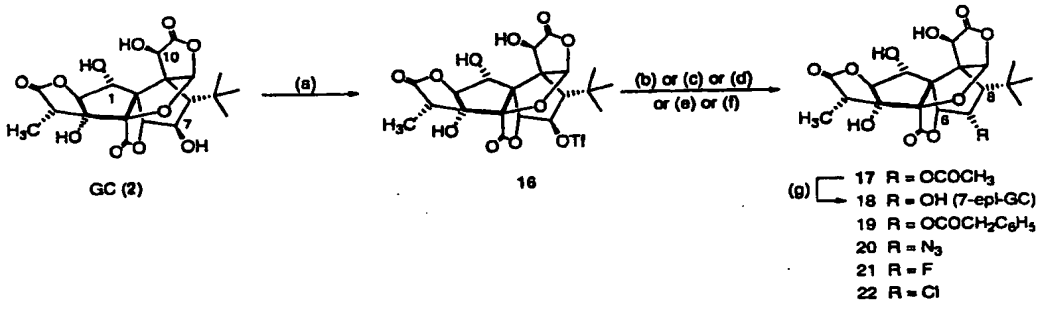
X = leaving group
R = any alkyl, phenyl etc. group

Methods

SYNTHESIS

For the synthesis of derivatives with variation at C-7, a useful intermediate was 7β-OTf-GB (**16**). GC (**2**) reacted with high selectivity at 7-OH with trifluoromethanesulfonic (Tf) anhydride giving **16** in very high yield, with no reactions occurring at other hydroxyl groups (Teng, B.-P., U.S. Patent No. 5,599,950). This selectivity is noteworthy, as 10-OH, and in some cases 1-OH, of GC (**2**) is generally the more reactive hydroxyl group (U.S. Patent No. 5,541,183; Hu, 2000), although we recently observed higher reactivity of 7-OH when acetylation was performed under strong acidic conditions (Jaracz, 2002).

Scheme 1

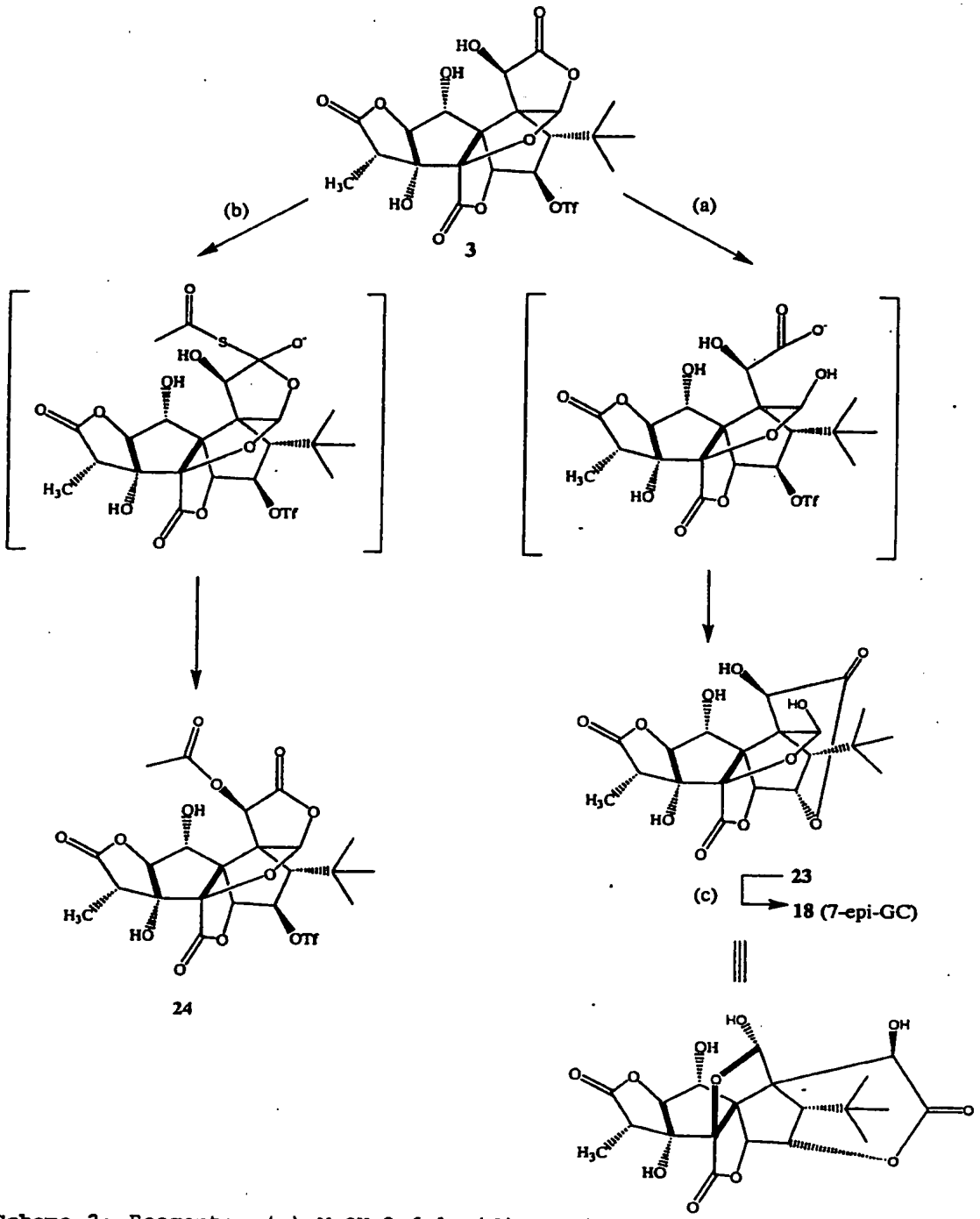


Scheme 1. Reagents: (a) Tf_2O, Pyridine, CH_2Cl_2; (b) $NaOCOCH_3$, DMSO; (c) $NaOCOCH_2C_6H_5$, DMSO; (d) NaN_3, DMSO; (e) TBAF, CH_3CN; (f) TBACl, CH_3CN ; (g) 2N NaOH.

7β-OTf-GB (**16**) was reacted with various nucleophiles as depicted in Scheme 1 to give derivatives **17-22**. The inverted configuration at C-7 was reflected by considerable changes in coupling constants in ^1H NMR spectra, i.e., $^3J_{7,8}$ and $^3J_{6,7}$ are

12 and 4 Hz in GC (**2**), whereas they are 3-5 Hz and ca. 0 Hz, respectively, when the configuration at C-7 is inverted. The reactions shown in Scheme 1 generally proceeded in good yield, but in several other cases the nucleophilic substitution did not proceed as expected. When reacting with a soft nucleophile such as NaSCN only starting material was recovered. Increase in the basicity of the nucleophiles, as in NaCN and aliphatic amines, resulted in a complex mixture of products, probably due to reaction at C-11, as previously described (Hu, 2001). In addition to the presence of multiple electrophilic sites in **16** it is believed that the steric hindrance of the bulky *tert*-butyl group, which is in close proximity to the reaction site, is responsible for lack of reaction. This assumption is corroborated by reaction of **16** with halogens; incorporation of fluorine (7α-F-GB, **21**) proceeded in high yield, chlorine (7α-Cl-GB, **22**) in slightly lower yield, whereas the larger bromine was introduced in trace amounts only, while no iodine product could be detected. These results were not affected by changing the solvent or the halide counterion.

Scheme 2

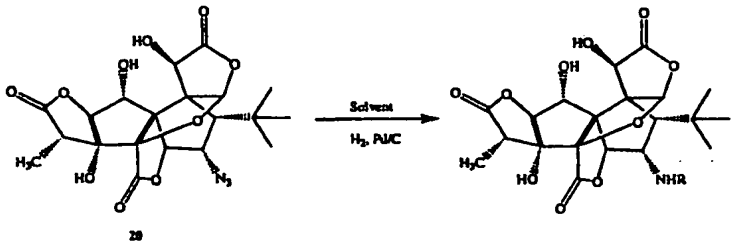


Scheme 2: Reagents: (a) MeOH,2,6-lutidine; (b) $KSCOCH_3$ DMF; (c) 1 M NaOH, acidic work-up

Steric hindrance may also be the prerequisite for two remarkable products arising from reaction of triflate **16** with

MeOH and $NaSCOCH_3$, respectively (Scheme 2). In the former case **16** was dissolved in MeOH and 2,6-lutidine, and reacted for three days at 70 °C expecting to provide 7α-OMe-GB; instead a new product with a molecular weight similar to GC (**2**), but with a different ^1H NMR spectrum was obtained. Extensive NMR studies revealed a new relactonized structure, neoginkgolide C (**23**) (Scheme 2), arising from opening of lactone C, followed by displacement of the triflate group. The structure of this compound **23** with a new rearranged ginkgolide skeleton not encountered earlier was determined by high resolution mass spectrometry, rotating frame nuclear Overhauser and exchange spectroscopy (ROESY), correlation spectroscopy (COSY) and heteronuclear single-quantum coherence (HSQC) NMR experiments (see Supporting Information). Moreover, treatment of compound neoginkgolide C (**23**) with 1 M NaOH followed by acidic work-up resulted in a clean conversion to the thermodynamically more favorable 7-epi-GC (**18**). The reaction between triflate **16** and $NaSCOCH_3$ did not give the expected 7α-$SCOCH_3$-GB, but instead the 10-acetate, while the 7-triflate group remained intact to give **24** (Scheme 2). This product might arise from a reaction by thioacetate at C-11, followed by a transfer of the acetate to 10-OH, tautomerization to thionic acid and relactonization to give the final product (Scheme 2).

Table 3. Reduction of 7α-N_3-GC (**20**) in different solvents.



20

Compound	Solvent	R	Yield (%)[a]
25	MeOH	CH_3	95
26	EtOH	CH_2CH_3	68
27	THF	H	98

[a]Isolated yield (after flash chromatography).

Another interesting feature was the reduction of azide **20** using Pd/C in MeOH under hydrogen. The reaction did not provide the expected amine, but instead gave *N*-methylamine **25** in quantitative yield (Table 3). To investigate this further, the reaction was carried out in EtOH, which gave *N*-ethyl amine **26** as the major product. The desired primary amine **27** was obtained when THF was used as solvent (Table 3). This intriguing reaction might provide a convenient way to convert azides directly into various alkylamines, an aspect which is under further investigation.

For the synthesis of 7-epi-GC (**18**) (Scheme 1) various approaches were attempted; GC (**2**) and 4-nitrobenzoic acid was treated with diethyl azodicarboxylate (DEAD) and Ph_3P in a Mitsunobu reaction, but no reaction was observed. Instead, 7β-OTf-GB (**16**) was reacted with KNO_2 and 18-crown-6 ether in a reaction that could potentially lead to 7-epi-GC (**18**) directly from **16** (Moriarty, 1993), but only starting material was recovered. Instead, the inversion of 7-OH of GC (**2**) was

accomplished using acetate as the nucleophile, followed by basic hydrolysis of the acetate. Acetylation of 7β-OTf-GB (**16**) was achieved by reaction with NaOAc; attempts to use the more reactive CsOAc led to decomposition of **16**, while using $CsOCCF_3$ did not lead to any reaction. The hydrolysis was accomplished by treating **17** with 2N NaOH to give 7-epi-GC (**18**) in 95% yield (Scheme 1).

To further investigate the importance of stereochemistry at C-7, we planned a series of corresponding 7β-substituted derivatives. Thus 7-epi-GC (**18**) was reacted with Tf anhydride, but only starting material was recovered. This lack of reaction is most likely due to a change in steric environment of 7α-OH, relative to 7β-OH, due to the *tert*-butyl group.

Finally, the observation that an aromatic substituent at 10-OH of GB (**1**) and GC (**2**) increases the antagonistic effect at PAFR (U.S. Patent No. 5,541,183; Hu, 2000; Strømgaard, 2002) led us to investigate whether a similar increase would be observed for 7α-GB derivatives. Benzylated derivatives **28-31** (Table 5) were therefore prepared, following previously described procedures (U.S. Patent No. 5,541,183; Hu, 2000).

Detailed synthetic procedure

GB (**1**) and GC (**2**) was obtained by extraction of leaves from *G. biloba*, purification by column chromatography and recrystallization as previously described (Lichtblau, 2002; van Beek, 1997). The purity was > 98% as estimated by ^1H NMR. Unless otherwise noted, materials were obtained from a commercial supplier and were used without further purification. Solvents were dried by eluting through alumina columns. Flash column chromatography was performed using ICN silica gel (32-63 mesh). Thin-layer chromatography was

carried out using pre-coated silica gel 60 F_{254} plates with thickness of 0.25 mm. Plates were heated and spots were detected by monitoring at 254 nm. ^1H and ^{13}C NMR spectra were obtained on Bruker DMX 300 MHz or Bruker DMX 400 MHz spectrometers and are reported in parts per million (ppm) relative to internal solvent signal, with coupling constants (*J*) in Hertz (Hz). HSQC, COSY and ROESY spectra were obtained on a Bruker DMX 400 MHz or Bruker DMX 500 MHz spectrometer. Analytical and preparative high performance liquid chromatography (HPLC) were performed on a HP 1100 LC instrument with detection by UV at 219 and 254 nm. Preparative HPLC was performed using a 10 μm C18 reversed-phase VYDAC column (250 × 22 mm) with a flow of 4 ml/min and eluting with either eluent A or B. A: water/CH_3CN/TFA (60:40:0.1), raising to (40:60:1) after 20 min. B: water/CH_3CN/TFA (65:35:0.1), raising to (40:60:1) after 20 min. Analytical HPLC were performed using a 5 μm C18 reversed-phase Phenomenex Luna column (150 x 4.60 mm), with a flow of .1 mL/min eluting with water/CH_3CN/TFA 70:30:0.1. Compounds **18** and **23** were eluted with water/CH_3CN/TFA 80:20:0.10 and compounds **25-27** with water/CH_3CN 90:10. Accurate mass determinations were performed on a JEOL JMS-HX110/100A HF mass spectrometer using a 3-nitrobenzyl alcohol (NBA) matrix and Xe ionizing gas, and are within ± 10 ppm of theoretical values. All were crystalline compounds that decompose above 200°C.

Example 7
7-Trifluoromethanesulfonyloxy Ginkgolide B (16). In a mixture of dry CH_2Cl_2 (1.0 mL) and dry pyridine (1.5 mL) GC (**2**) (184 mg, 0.42 mmol) was dissolved. The solution was cooled to −20 °C under argon and trifluoromethanesulfonic anhydride (78 μL) was added dropwise. The reaction was stirred at −20 °C for 2 h and allowed to warm to room temperature over 1 h. The solvent

was removed in vacuo, and the residue was dissolved in EtOAc (30 mL) and washed with 1 N HCl (3 × 20 mL), brine (10 mL), dried ($MgSO_4$) and the solvent removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3/CH_3OH$/EtOAc (30:1:1 and 20:1:1) to obtain **3** as white crystals (232 mg, 97%). ^1H NMR (400 MHz, DMSO-d_6): δ 1.11 (s, *tert*-butyl), 1.13 (d, J = 9.6, CH_3), 2.22 (d, J = 16.5, 8-H), 2.82 (q, J = 9.6, 14-H), 4.15 (dd, J = 8.0, 5.9, 1-H), 4.73 (d, J = 8.0, 2-H), 5.08 (d, J = 7.4, 10-H), 5.24 (dd, J = 16.5, 5.6, 7-H) 5.41 (d, J = 5.6, 6-H), 5.54 (d, J = 5.9, 1-OH), 6.20 (s, 12-H), 6.62 (s, 3-OH), 7.63 (d, J = 7.4, 10-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.1, 29.2 (3C), 32.6, 42.1, 49.0, 64.2, 68.1, 69.4, 74.4, 75.2, 84.0, 86.3, 93.2, 99.9, 109.4, 118.6 (q, $^1J_{CF}$ = 316.6, CF_3), 173.9, 176.9, 179.2. HRMS: $C_{21}H_{23}F_3O_{13}S$ requires M + 1 at m/z 573.0890; found, 573.0872.

Example 8

7α-*O*-Acetate Ginkgolide B (17). Sodium acetate (163 mg, 1.99 mmol) and **16** (228 mg, 0.39 mmol) were dissolved in DMSO (3 mL) and the solution was stirred at 65 °C for 17 h. The solvent was removed *in vacuo*, and the residue was partitioned between 1 N HCl (20 mL) and EtOAc (25 mL). The aqueous phase was extracted with EtOAc (3 × 25 mL) and the combined organic phases were washed with brine (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/EtOAc/MeOH (10:1:1) to obtain **17** as white crystals (144 mg, 75%). A portion (20 mg) of this was recrystallized (MeOH/H_2O) for pharmacological evaluation (9 mg). ^1H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.1, CH_3), 1.10 (s, *tert*-butyl), 1.91 (d, J = 3.3, 8-H), 2.06 (s, $COCH_3$), 2.84 (q, J = 7.1, 14-H), 4.03 (dd, J = 7.7, 3.6, 1-H), 4.66 (d, J = 7.7, 2-H), 4.86 (d, J = 3.6, 1-OH), 5.01 (s, 6-H), 5.15 (d, J = 6.8, 10-H), 5.25 (d, J = 3.3, 7-H),

6.16 (s, 12-H), 6.52 (s, 3-OH), 7.42 (d, J = 6.8, 10-OH). ^{13}C NMR (100 MHz, CD_3OD): δ 8.0, 21.0, 30.7 (3C), 33.8, 43.4, 53.2, 70.1, 70.3, 72.5, 75.3, 79.6, 80.9, 84.6, 92.6, 99.8, 112.7, 171.3, 171.5, 175.0, 178.1. HPLC-UV: 96%. HRMS: $C_{22}H_{27}O_{12}$ requires M + 1 at m/z 483.1503; found, 483.1525.

Example 9

7α-*O*-Phenylacetate Ginkgolide B (19). Sodium phenylacetate (44 mg, 0.28 mmol) and **16** (32 mg, 0.07 mmol) were dissolved in DMSO (0.8 mL) and heated at 65 °C for 5 h and the solvent was removed in vacuo, and the residue was partitioned between 1 N HCl (10 mL) and EtOAc (15 mL) and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with 1 N HCl (2 × 10 mL), water (5 × 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$) and the solvent removed in vacuo. The crude product was purified by flash column chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1), recrystallized (MeOH) and further purified by preparative HPLC (eluent A) to give 5 (10 mg, 26%) as white crystals. ^1H NMR (400 MHz, DMSO-d_6): δ 1.07 (s, *tert*-butyl), 1.12 (d, J = 6.9, CH_3), 1.93 (d, J = 2.9, 8-H), 2.84 (q, J = 6.9, 14-H), 3.70 (s, CH_2), 4.04 (dd, J = 3.6, 7.7, 1-H), 4.62 (d, J = 7.7, 2-H), 4.72 (d, J = 3.6, 1-OH), 4.99 (s, 6-H), 5.16 (d, J = 6.6, 10-H), 5.26 (d, J = 2.9, 7-H), 6.16 (s, 12-H), 6.48 (s, 3-OH), 7.25-7.35 (m, aromatic, 5H) 7.46 (d, J = 6.6, 10-OH). ^{13}C NMR (100 MHz, CD_3OD): δ 8.0, 30.8 (3C), 33.8, 42.2, 43.4, 53.3, 70.1, 70.3, 72.5, 75.2, 80.2, 81.0, 84.6, 92.6, 99.9, 112.7, 128.4, 129.7, 130.5, 134.7, 171.4, 172.2, 175.0, 178.1. HPLC-UV: 98%. HRMS: $C_{28}H_{31}O_{12}$ requires M + H at m/z 559.1816; found, 559.1826.

Example 10

7α-Azido Ginkgolide B (20). Sodium azide (87 mg, 1.34 mmol)

and **16** (153 mg, 0.27 mmol) were dissolved in DMSO (2.5 mL) and the solution was heated at 65 °C for 26 h. The solvent was removed *in vacuo*. The solid was partitioned between saturated aqueous NH_4Cl (20 mL) and EtOAc (20 mL) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give the **20** as white crystals (109 mg, 88%). A portion (23 mg) of this was recrystallized (MeOH/H_2O) for pharmacological evaluation (12 mg). ^1H NMR (300 MHz, DMSO-d_6): δ 1.12 (d, J = 7.1, CH_3), 1.13 (s, tert-butyl), 1.80 (d, J = 4.0, 8-H), 2.73 (q, J = 7.1, 14-H), 4.05 (dd, J = 7.6, 3.6, 1-H), 4.70 (d, J = 7.6, 2-H), 4.74 (d, J = 4.0, 7-H), 4.97 (d , J = 3.6, 1-OH), 5.06 (d, J = 6.0, 10-H), 5.25 (s, 6-H), 6.10 (s, 12-H), 6.52 (s, 3-OH), 7.05 (d, J = 6.0, 10-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.8, 30.1 (3C), 32.7, 41.6, 51.6, 67.2, 68.4, 68.5, 71.0, 73.7, 79.6, 82.9, 92.9, 98.2, 110.3, 169.5, 173.4, 176.2. HPLC-UV: 99%. HRMS: $C_{20}H_{24}O_{10}N_3$ requires M + 1 at m/z 466.1462; found, 466.1445.

Example 11

7α-Fluoro Ginkgolide B (21). Tetrabutylammonium fluoride hydrate (37 mg, 0.14 mmol) and **16** (62 mg, 0.11 mmol) were dissolved in CH_3CN (1 mL) and heated at 80 °C for 1.5 h. The solvent was removed in vacuo, and the residue was partitioned between 1 N HCl (10 mL) and EtOAc (15 mL) and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (2 × 15 mL), brine (2 × 15 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by flash column chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) followed by preparative HPLC (eluent B) to give **21** as white crystals (34 mg, 71%). ^1H

NMR (400 MHz, CD_3OD): δ 1.25 (s, *tert*-butyl), 1.26 (d, J = 7.1, CH_3), 1.94 (dd, $^2J_{HF}$ = 45.5, J = 2.3, 8-H), 3.05 (q, J = 7.1, 14-H), 4.24 (d, J = 8.0, 1-H), 4.59 (d, J = 8.0, 2-H), 5.18 (s, 10-H), 5.35 (d, $^2J_{HF}$ = 10.9, 6-H), 5.38 (dd, $^1J_{HF}$ = 48.8, J = 2.3, 7-H), 6.14 (s, 12-H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.0, 29.5 (3C), 32.9, 42.4, 53.7 ($^2J_{CF}$ = 20.4 Hz), 68.8, 69.1, 71.5, 74.5, 79.5 ($^2J_{CF}$ = 36.0 Hz), 83.7, 91.7, 96.9 ($^1J_{CF}$ = 184.2 Hz), 98.8, 111.6, 171.2, 173.9, 177.1. HRMS: $C_{20}H_{23}FO_{10}$ requires M + 1 at *m/z* 443.1354; found, 443.1370.

Example 12

7α-Chloro Ginkgolide B (22). Tetrabutylammonium chloride (86 mg, 0.31 mmol) and **16** (36 mg, 0.06 mmol) were dissolved in CH_3CN (1.4 mL) and heated at 80 °C for 12 h. The solvent was removed in vacuo and the residue partitioned between 1 N HCl (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with water (4 × 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by preparative HPLC (eluent B) and recrystallized ($CH_3CN/CHCl_3$) to give **22** as white crystals (9 mg, 30%). ^1H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.0, CH_3), 1.17 (s, *tert*-butyl), 2.19 (d, J = 4.2, 8-H), 2.85 (q, J = 7.0, 14-H), 4.03 (dd, J = 7.7, 3.3, 1-H), 4.63 (d, J = 3.3, 1-OH), 4.68 (d, J = 7.8, 2-H), 4.84 (d , J = 4.2, 7-H), 5.13 (d, J = 6.4, 10-H), 5.26 (s, 6-H), 6.17 (s, 12-H), 6.53 (s, 3-OH), 7.57 (d, J = 6.4, 10-OH). ^{13}C NMR (100 MHz, CD_3OD): δ 8.7, 31.2 (3C), 34.5, 42.5, 54.2, 65.1, 69.0, 70.0, 72.2, 74.4, 83.7, 84.2, 90.7, 99.4, 111.3, 169.9, 174.4, 177.1. HPLC-UV: 98%. HRMS: $C_{20}H_{24}O_{10}Cl$ requires M + 1 at m/z 459.1058; found, 459.1052.

Example 13

Neoginkgolide C (23). Triflate **16** (27 mg, 0.05 mmol) was dissolved in dry MeOH (470 µL) and 2,6-lutidine (150 µL) was added and the reaction mixture was heated at 65 °C for 3 days. The solvent was removed in vacuo and the residue purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) to give the crude product, which was further purified by preparative HPLC (eluent A) to give **23** as white crystals (6 mg, 29%). ^1H NMR (400 MHz, CD_3OD): δ 1.20 (m, CH_3 and *tert*-butyl), 1.64 (dd, *J* = 1.4, 1.2, 8-H), 3.72 (q, *J* = 7.1, 14-H), 4.46 (d, *J* = 8.0, 2-H), 4.59 (d, *J* = 1.2, 10-H), 4.72 (d, *J* = 8.0, 1-H), 5.00 (dd, *J* = 1.4, 1.3, 7-H), 5.14 (d, *J* = 1.3, 6-H), 5.96 (s, 12-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.6, 30.2 (3C), 32.7, 41.3, 47.8, 60.2, 66.9, 67.8, 73.6, 75.6, 82.5, 83.4, 92.7, 93.7, 104.9, 170.2, 171.7, 177.6. HPLC-UV: 98%. HRMS: $C_{20}H_{24}O_{11}$ requires M + Na at m/z 463.1216; found, 463.1245.

Example 14

10-*O*-Acetate-7-trifluoromethanesulfonyloxy Ginkgolide B (24). Potassium thioacetate (4 mg, 0.035 mmol) and **16** (3 mg, 0.006 mmol) were dissolved in dry DMF (35 µl) and heated at 40 °C for 3 h. The solvent was removed in vacuo, and the residue was partitioned between water (10 mL) and EtOAc (15 mL) and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (5 × 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) to give **24** (1.3 mg, 18%) as white crystals. ^1H NMR (400 MHz, DMSO-d_6): δ 1.08 (s, *tert*-butyl), 1.13 (d, *J* = 7.1, CH_3), 2.21 (s, $COCH_3$), 2.31 (d, *J* = 12.6, 8-H), 2.85 (q, *J* = 7.1, 14-H), 4.08 (dd, *J* = 5.9, 5.8, 1-H), 4.75 (d, *J* = 5.9, 2-H), 5.09 (dd, *J*

= 12.6, 4.2, 7-H), 5.48 (d, J = 4.2, 6-H), 6.13 (s, 10-H), 6.33 (s, 12-H), 6.53 (s, 3-OH), 6.71 (d, J = 5.8, 1-OH). HRMS: $C_{23}H_{26}O_{14}F_3S$ requires M + 1 at m/z 615.0995; found, 615.1016.

Example 15

7α-*N*-Methylamino Ginkgolide B (25). Azide **20** (38 mg, 0.08 mmol) was dissolved in dry MeOH (1.2 ml) and Pd/C (10%, 12 mg) was added. The suspension was stirred under an atmosphere of H_2 for 48 h. The solvent was removed in vacuo and EtOAc (10 mL) was added and the solution filtered through Celite. The solvent was removed in vacuo, and the crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give white crystals, which were recrystallized (MeOH) to give **25** (23 mg, 65%) as white crystals. ^{1}H NMR (400 MHz, CD_3OD): δ 1.22 (m, *tert*-butyl and CH_3), 1.89 (d, J = 4.4, 8-H), 2.47 (s, CH_3), 3.06 (q, J = 7.0, 14-H), 3.46 (d, J = 4.4, 7-H), 4.24 (d, J = 7.2, 1-H), 4.53 (d, J = 7.2, 2-H), 5.05 (s, 6-H), 5.31 (s, 10-H), 6.17 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.2, 31.3 (3C), 33.4, 34.3, 43.3, 53.5, 69.1, 69.6, 70.8, 72.6, 75.4, 79.3, 84.4, 94.5, 100.6, 112.2, 172.6, 174.8, 178.4. HPLC-UV: 97%. HRMS: $C_{21}H_{28}O_{10}N$ requires M + 1 at m/z 454.1713; found, 454.1719.

Example 16

7α-*N*-Ethylamino Ginkgolide B (26). Azide **20** (48 mg, 0.10 mmol) was dissolved in dry EtOH (1.0 mL) and Pd/C (10%, 15 mg) was added. The suspension was stirred under an atmosphere of H_2 for 48 h. The solvent was removed in vacuo and EtOAc (10 mL) was added and the solution filtered through Celite. The solvent was removed in vacuo and the residue purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give white crystals, which were recrystallized (MeOH) to give

26 (22 mg, 47%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.11 (t, J = 7.1, CH_3), 1.23 (m, *tert*-butyl and CH_3), 1.89 (d, J = 4.5, 8-H), 2.57 (dq, J = 7.1, 12.0, CH_2, 1H), 2.94 (dq, J = 7.1, 12.0, CH_2, 1H), 3.06 (q, J = 7.1, 14-H), 3.56 (d, J = 4.5, 7-H), 4.22 (d, J = 7.3, 1-H), 4.53 (d, J = 7.3, 2-H), 5.08 (s, 6-H), 5.27 (s, 10-H), 6.16 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.2, 15.5, 31.3 (3C), 34.4, 41.6, 43.3, 53.5, 67.5, 69.2, 70.8, 72.6, 75.3, 80.0, 84.4, 94.4, 100.6, 112.3, 172.6, 174.9, 178.4. HPLC-UV: 98%. HRMS: $C_{22}H_{29}O_{10}N$ requires M + 1 at m/z 468.1870; found, 468.1867.

Example 17

7α-Amino Ginkgolide B (27). Azide **20** (10 mg, 0.02 mmol) was dissolved in dry THF (0.4 mL) and Pd/C (10%, 8 mg) was added. The suspension was stirred under an atmosphere of H_2 for 14 h. EtOAc (10 mL) was added and the solution filtered through Celite. The solvent was removed in vacuo to give white crystals which were recrystallized (MeOH) to give **27** as white crystals (5 mg, 49%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.20 (s, *tert*-butyl), 1.23 (d, J = 7.1, CH_3), 1.90 (d, J = 3.2, 8-H), 3.08 (q, J = 7.1, 14-H), 3.83 (d, J = 3.2, 7-H), 4.26 (d, J = 7.0, 1-H), 4.51 (d, J = 7.0, 2-H), 5.01 (s, 6-H), 5.04 (s, 10-H), 6.18 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.3, 31.0 (3C), 34.1, 43.2, 54.7, 60.3, 68.9, 70.9, 72.6, 74.9, 83.9, 84.4, 95.0, 100.3, 111.8, 172.3, 174.4, 178.4. HPLC-UV: 97%. HRMS: $C_{42}H_{26}O_{10}N$ requires M + H at m/z 440.1557; found, 440.1594.

Example 18

7-Epi-ginkgolide C (18). Acetate **17** (42 mg, 0.087 mmol) was dissolved in a mixture of MeOH and 2 N NaOH (2:1, 1.8 mL) and stirred for 5 h. 1 N HCl was added and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases

were washed with brine (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo to give **18** (36 mg, 95%) as white crystals. A portion (15 mg) of this was recrystallized ($MeOH/H_2O$) for pharmacological evaluation (6 mg). ^1H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.0, CH_3), 1.12 (s, *tert*-butyl), 1.64 (d, J = 2.7, 8-H), 2.89 (q, J = 7.0, 14-H), 4.11 (dd, J = 4.5, 6.8, 1-H), 4.37 (dd, J = 2.7, 6.3, 7-H), 4.59 (d, J = 6.8, 2-H), 4.98 (s, 6-H), 5.04 (d, J = 2.7, 10-H), 5.52 (d , J = 6.3, 7-OH), 5.64 (d, J = 4.5, 1-OH), 6.13 (s, 12-H), 6.45 (s, 3-OH), 6.73 (d, J = 2.7, 10-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.0, 30.2 (3C), 32.6, 41.4, 52.3, 68.8, 69.6, 71.5, 73.4, 76.2, 80.8, 82.8, 92.9, 98.6, 109.7, 170.0, 172.7, 176.3. HPLC-UV: 98%. HRMS: $C_{20}H_{25}O_{11}$ requires M + 1 at m/z 441.1397; found, 441.1395.

Example 19

10-*O*-Benzyl Ginkgolide B (28). Synthesis and analytical data as previously described (Park, P.-U., *et al.*; Hu, L., *et al.*, **2000**).

Example 20

10-*O*-Benzyl Ginkgolide C (29). K_2CO_3 (31 mg, 0.22 mmol) was added to a solution of **2** (12 mg, 0.02 mmol) dissolved in DMF (0.2 mL) followed by addition of benzyl chloride (30 μL, 0.26 mmol). The suspension was stirred for 2.5 h at 60 °C. The solvent was removed in vacuo, and the residue partitioned between 1 N HCl (10 mL) and EtOAc (15 mL) and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (2 × 10 mL) and brine NaCl (2 × 10 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) and further by preparative HPLC (solvent system A) to give **16** (9 mg, 77%) as

white crystals. ^{1}H NMR (400 MHz, CD_3OD): δ 1.21 (s, *tert*-butyl), 1.23 (d, *J* = 7.1, CH_3), 1.76 (d, *J* = 12.5, 8-H), 3.01 (q, *J* = 7.1, 14-H), 4.13 (dd, *J* = 12.3, 4.3, 7-H), 4.19 (d, *J* = 7.4, 1-H), 4.49 (d, *J* = 7.4, 2-H), 4.76 (d, *J* = 10,2, CH_2, 1H), 5.04 (s, 6-H), 5.02 (d, *J* = .4.3, 6-H), 5.25 (s, 10-H), 5.46 (d, *J* = 10.2, CH_2, 1H), 6.14 (s, 12-H), 7.37-7.44 (m, aromatic, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.2, 29.1 (3C), 32.2, 41.6, 50.5, 64.1, 67.1, 73.8, 74.3, 75.6, 77.2, 79.3, 83.5, 90.6, 98.5, 110.1, 128.9 (2C), 129.5 (2C), 129.8, 134.2, 170.8, 170.8, 175.5. HPLC-UV: 98%. HRMS: $C_{27}H_{30}O_{11}$ requires M + Na at m/z 553.1686; found, 553.1684.

Example 21

10-*O*-Benzyl-7α-fluoro Ginkgolide B (30). Synthesized as described for **29** using K_2CO_3 (107 mg, 0.77 mmol), **21** (34 mg, 0.08 mmol), and benzyl chloride (89 μL, 0.77 mmol) in DMF (1.8 mL). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give **30** (16 mg, 39%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (s, *tert*-butyl), 1.30 (d, *J* = 7.0, CH_3), 1.88 (dd, $^2J_{HF}$ = 44.5, *J* = 2.3, 8-H), 2.94 (d, *J* = 3.1, 1-OH), 3.06 (q, *J* = 7.0, 14-H), 4.28 (dd, *J* = 8.1, 3.1, 1-H), 4.50 (d, *J* = 8.1, 2-H), 4.68 (d, *J* = 9.4, CH_2, 1H), 4.95 (s, 10-H), 5.29 (d, $^2J_{HF}$ = 10.2, 6-H), 5.30 (dd, $^1J_{HF}$ = 50.1, *J* = 1.5, 7-H), 5.41 (d, *J* = 9.4, CH_2, 1H), 6.04 (s, 12-H), 7.36 (m, aromatic, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.2, 30.3 (3C), 32.9, 41.7, 53.3 ($^2J_{CF}$ = 20.4), 68.2, 71.6, 74.1, 74.4, 75.1, 79.5 ($^2J_{CF}$ = 35.6), 83.6, 90.2, 96.0 ($^1J_{CF}$ = 184.2), 98.2, 110.9, 128.7(2C), 129.1(2C), 129.3, 134.8, 170.2, 170.8, 175.1. HPLC-UV: 98%. HRMS: $C_{27}H_{30}O_{10}F$ requires M + 1 at m/z 533.1823; found, 533.1784.

Example 22

10-*O*-Benzyl-7-epi-ginkgolide C (31). Synthesized as described for **29** using K_2CO_3 (50 mg, 0.36 mmol), **18** (16 mg, 0.04 mmol), and benzyl chloride (42 μL, 0.36 mmol) in DMF (0.3 mL). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) and further by preparative HPLC (eluent A) to give **31** (11 mg, 56%) as white crystals. ^1H NMR (400 MHz, $CDCl_3$): δ 1.23 (s, *tert*-butyl), 1.29 (d, *J* = 7.0, CH_3), 1.83 (d, *J* = 3.1, 8-H), 2.60 (d, *J* = 3.6, 1-OH), 2.66 (d, *J* = 11.0, 7-OH), 3.06 (q, *J* = 7.0, 14-H), 3.40 (bs, 3-OH), 4.28 (dd, *J* = 3.6, 7.8, 1-H), 4.48 (m, 2-H, 7-H), 4.72 (d, *J* = 9.2, CH_2, 1H), 4.96 (s, 6-H), 5.51 (s, 10-H), 5.50 (d, *J* = 9.2, CH_2, 1H), 6.09 (s, 12-H), 7.39-7.44 (m, aromatic, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.3, 30.6 (3C), 33.1, 41.6, 52.9, 68.4, 71.3, 74.0, 74.4, 74.7, 77.6, 82.3, 83.2, 90.7, 98.5, 110.5, 129.2 (2C), 129.6 (2C), 130.1, 133.8, 170.3, 170.5, 175.4. HPLC-UV: 99%. HRMS: $C_{27}H_{31}O_{11}$ requires M + 1 at m/z 531.1866; found, 531.1895.

BIOLOGICAL TESTING - COMPOUNDS 1-15

Radioligand Binding Assay. The radioligand binding assays were performed as previously described (Shindou, 2000). In brief, membrane fractions from hearts and skeletal muscles of PAFR-Tg mice (50 µl suspension containing 121 fmol of PAFR) were mixed with 2 pmol of [^{3}H]-WEB in 50 µl of Buffer [25 mM Hepes/NaOH (pH 7.4), 0.25 M sucrose,10 mM $MgCl_2$, 0.1% BSA], and the compound to be tested in 100 µl of buffer in a 96-well microplate in triplicate for each concentration. These mixtures were incubated at 25°C for 90 min, upon which the receptor-bound [^{3}H]-WEB 2086 was filtered and washed with cold Buffer. The plates were then dried at 50 °C for at least 90 min., 25 µl of MicroScint-0 scintillation cocktail was added, and filters were placed in a TopCount microplate scintillation counter. Binding data were analyzed with the nonlinear curve-fitting program Microplate Manager III (Bio-Rad, Hercules, CA). Calculated IC_{50} values were then converted to K_i values using the Cheng-Prusoff correction (Cheng, 1973), with the following equation: $K_i = IC_{50}/(1 + [L]/K_D)$, where [L] is the concentration of the radioligand, and K_D is the previously determined dissociation constant for [^{3}H]-WEB 2086 (4.3 nM) (Shindou, 2000). Non-specific binding was determined using methods as previously described (Shindou, 2000).

RESULTS

Synthesis. A series of photoactivatable GB (**2**) and ginkgolide C (GC, **3**) derivatives were synthesized. The design of GB derivatives **8a-c** and GC derivatives **9a-c** (Figure 3) was based on previous SAR studies of ginkgolides which demonstrated that bulky aromatic substituents in the 10-OH position of GB (**2**) increases activity at the PAFR (Park, 1996; Hu, 1999; Hu, 2000). Three different photoactivatable moieties, benzophenone, trifluoromethyldiazirine and tetrafluorophenyl azide (see **7a-c**,

Figure 3) were chosen as they have been described as being among the most successful for labeling receptors and enzymes (Dorman, 2000; Flemming, 1995; Kotzyba-Hilbert, 1995). Most importantly, upon irradiation these photoactivatable groups react with the receptor via different intermediates, namely, a radical, a carbene or a (singlet) nitrene for the benzophenone (**7a**), trifluoromethyldiazirine (**7b**) and tetrafluorophenyl azide (**7c**) moieties, respectively (Dorman, 2000). Since it is essentially impossible to predict which group will be most readily incorporated into the receptor, use of these different groups increases the likelihood of a successful incorporation.

Preparation of GB derivatives **8a-c** and GC derivatives **9a-c** was performed by reacting GB (**2**) and GC (**3**) with 4-(bromomethyl)benzophenone (**7a**), 3-(4-bromomethylphenyl)-3-trifluoromethyl-3*H*-diazirine (**7b**) and 1-azido-4-(bromomethyl)-2,3,5,6-tetrafluorobenzene (**7c**), respectively (Figure 3). Benzophenone **7a** was commercially available, whereas trifluoromethyldiazirine **7b** (Nassal, 1983; Nassal, 1984) and tetrafluorophenyl azide **7c** (Keana, 1990; Lei, 1998; Lei, 2000) (Figure 5) were synthesized, respectively, in 3 and 7 steps essentially as previously described. Ginkgolides GB (**2**) and GC (**3**) were derivatized almost exclusively at 10-OH when potassium hydride (KH) was used as base, as was previously shown for GB (**2**) (Park, 1996), whereas other bases were less selective, giving rise to products derivatized at 1-OH as well. Generally, the position of the substituent was determined from the coupling systems of the appropriate protons in DMDO-d_6, as well as by COSY NMR spectra. The relative chemical shift of 12-H in DMSO-d_6 can also be used in differentiating 1- and 10-OH substitutions (Hu, 2000).

GC derivatives **9a-c** can be reacted further to incorporate fluorescent groups; for example, benzophenone derivative **9a** was

reacted with one equivalent of 5-(dimemethylamino)naphthalene-sulfonyl chloride (dansyl chloride), to give 10-*O*-benzophenone-7-*O*-dansyl GC (**10a**) with almost exclusive reaction at 7-OH (Figure 4). Interestingly, increasing the amount of dansyl chloride to two equivalents gave 10-*O*-benzophenone-1-*O*-dansyl GC (**10b**) as well as (**10a**) in a 1:1 ratio. The coupling of GB (**2**) with 4-benzoylbenzoic acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide HCl (EDC) and 4-dimethylaminopyridine (DMAP) occurred exclusively at 10-OH to give 10-benzophenonecarbonyl GC (**11**) in good yield (Figure 5). In 10-benzophenonecarbonyl GC (**11**) the photoactivatable benzophenone moiety, as in the case of **8a** and **9a**, is linked to the ginkgolide skeleton through an ester linkage. Upon incorporation into the receptor, the ester group can be aminolysed with a fluorescent amine such as 1-pyrenemethylamine, thus avoiding the use of radioactivity for photolabeling and sequencing (Li, 1999).

For positron emission tomography (PET) studies derivatives labeled with [^{18}F]- and [^{11}C] possessing half lives of 110 min and 20 min, respectively, will be used. In the present work, preparation of the corresponding non-radioactive analogs has been performed. Compound **13**, a 7-fluoro analog of GB (**2**) which can ultimately be labeled with [^{18}F], was prepared by nucleophilic substitution of 7-*O*-triflate intermediate **12** with tetrabutylammonium fluoride (TBAF) (Figure 6). As expected for nucleophilic substitution, NMR showed the relative stereochemistry at C-7 to be reversed compared to GC (**3**). Intermediate **12** was prepared by reaction of GC (**3**) with trifluoromethanesulphonic anhydride [$(CF_3SO_2)_2O$] with remarkable selectivity for the 7-OH, as no substitutions at other hydroxyl groups was observed (Teng, 1997). Two other potential PET-ligands, which can be labeled with [^{11}C], were prepared by selective reaction of the 10-OH of GB (**2**) with either methyl iodide or 2-bromoethyl fluoride to give 10-*O*-methyl GB (**14**) and

10-*O*-(2-fluoroethyl) GB (**15**), respectively (Figure 6). Upon reacting GB (**2**) with methyl iodide, reaction at 1-OH could not be avoided, the 10-OH:1-OH product ratio being highly sensitive to the use of the appropriate base, e.g., K_2CO_3 gave primarily the 10-substituted analog, whereas tetrabutylammonium hydroxide (TBAH) gave mainly the 1-substituted derivative.

Pharmacology. The native terpene trilactones (**1-6**), as well as ginkgolide derivatives **8a-c**, **9a-c**, **10a**, **10b**, **11** and **13-15** were tested for their ability to bind to PAFR using radioligand binding assays with membrane fractions from hearts and skeletal muscles of PAFR transgenic mice (Shindou, 2000). Initially compounds were tested in concentrations of 5 μM against [^{3}H]-WEB 2086 (Fig. 2), a potent, competitive PAFR antagonist and [^{3}H]-PAF; the compounds were generally less potent against [^{3}H]-PAF, but the relative potencies were comparable with the two radioligands. The degree of non-specific binding was determined to be ca. 50% for [^{3}H]-PAF and less than 5% for [^{3}H]-WEB 2086. Accordingly, the assays were performed using [^{3}H]-WEB 2086 rather than [^{3}H]-PAF as the radioligand, mainly due to the high degree of non-specific binding of the latter.

All compounds were dissolved in DMSO to obtain 5 mM stock solutions of test compounds. Examination of the effect of DMSO on the binding of [^{3}H]-WEB 2086 revealed that up to 1% DMSO (final concentration) was acceptable, but 1-2.5% resulted in a slight inhibition of [^{3}H]-WEB 2086 binding. Generally this caused no problem; however, with very weakly binding compounds the relatively high DMSO concentration in solutions above 100 μM had a small inhibitory effect, thus leading to a slight overestimation of their potencies. Previous studies have reported problems specifically associated with the solubilization of ginkgolides in DMSO (Maclennan, 1996), but similar problems were not observed in the present study.

Native ginkgolides (**1-5**) and bilobalide (**6**) were tested with the cloned PAFR (Fig. 3*A* and Table 1). GB (**2**) was the most potent compound with a K_i value of 0.56 μM, while GA (**1**) was slightly less potent with a K_i of 1.46 μM. GC (**3**) and ginkgolide J (GJ, **4**) were significantly less potent, while ginkgolide M (GM, **5**) and bilobalide (**6**) both had K_i values larger than 50 μM. The GB-derived photoactivatable compounds **8a-c** and **11** with K_i values in the range 0.09-0.15 μM (Fig. 3*B* and Table 2) were all more potent than GB (**2**), while compounds **9a-c** derived from GC (**3**) with K_i values of 0.47-0.79 μM were equipotent to GB (**2**) (Fig. 3*C* and Table 2), despite the fact that GC (**3**) itself is only weakly potent. Besides proving that aromatic groups linked to 10-OH enhance activity in both GB (**2**) and GC (**3**) derivatives, these results also indicate that the specific type of photoactivatable group was less important. Derivatives **10a** and **10b** possessing a fluorescent dansyl group at either 1- or 7-OH were both less potent than 10-*O*-benzophenone GC (**9a**) without the dansyl group (Fig. 3*D*). However, an important difference was observed in the activities of the two; the 1- and 10-disubstituted analog (**10b**) was ca. four times more potent than the 7- and 10-disubstituted analog (**10a**) (Table 2).

Table 1. The K_i values of the native terpene trilactones.

Compound	K_i (μM)[a]	Compound	K_i (μM)[a]
GA (1)	1.46	GJ (4)	9.90
GB (2)	0.56	GM (5)	>50
GC (3)	12.6	BB (6)	>50

[a]Inhibition of [^{3}H]-WEB 2086 binding.

Table 2. The K_i values of the synthesized derivatives.

Compound	K_i (μM)[a]	Compound	K_i (μM)[a]
8a	0.15	**10a**	3.94
8b	0.15	**10b**	0.96
8c	0.09	**11**	0.13
9a	0.58	**13**	0.99
9b	0.47	**14**	3.16
9c	0.79	**15**	4.87

[a]Inhibition of [^{3}H]-WEB 2086 binding.

Finally the potential PET analogs **13-15** were tested. The fluorinated analog **13** had a K_i value of 0.99 μM (Table 2), thus being almost equipotent with the native compound GB (**2**). The C-10 derivatized compounds 10-*O*-methyl GB (**14**) and 10-*O*-(2-fluoroethyl) GB (**15**) were both significantly less potent than GB (**2**) with K_i values of 3.16 and 4.87 μM for **14** and **15**, respectively (Table 2).

DISCUSSION

Nine analogs (**8a-c**, **9a-c**, **10a**, **10b** and **11**) with photoactivatable groups, and in the case of **10a** and **10b** with fluorescent dansyl groups as well, have been prepared from native ginkgolides GB (**2**) and GC (**3**) by selective derivatizations of the hydroxyl groups. Furthermore, we have prepared three analogs (**13-15**), the radioactive versions of which will be used for PET studies. For the synthesis of 7-O-triflate intermediate **12**, reaction of GC (**3**) with sulfonic anhydride gave rise to remarkable selectivity at 7-OH (Teng, 1997). 10-*O*-Methyl GB (**14**) and 10-*O*-(2-fluoroethyl) GB (**15**) were synthesized by derivatization of GB (**2**), indicating that when the appropriate combination of alkylating agent and base is used, even small aliphatic groups react preferentially at the 10-OH. Generally, the increased reactivity of the 1-OH and 10-OH compared to 7-OH , has been rationalized by hydrogen-bonding between 1-OH and 10-OH (Corey, 1992), but this does not explain the interesting selectivity for the 10-OH position in reactions with benzyl bromide derivatives. Notably, reaction of GC (**3**) with a bulky silyl chloride protection group occurs exclusively at the 1-OH of GC (Weinges, 1991).

All native terpene trilactones as well as the derivatized compounds were investigated with respect to their binding to cloned PAFR isolated from transgenic mice (Fig. 3A). Previous SAR studies of PAFR antagonism with terpene trilactones and derivatives was performed by monitoring inhibition of PAF-induced rabbit platelet aggregation. GB (**2**) has generally been reported to be a potent antagonist of the PAFR based on the latter assay with an IC_{50} value around 0.2 μM (Park, 1996; Hu, 1999; Hu, 2000).

GM (**5**) has only been found in the root bark of the *G. biloba*

tree (Nakanishi, 1967) and is not readily available. Thus, the interaction between PAFR and GM (**5**) has not previously been reported. The remaining terpene trilactones are all found in the leaf of *G. biloba*. However **5**, lacking the hydroxyl group at C-3, was devoid of PAFR binding at the concentrations tested. Generally the activities of GC (**3**), GJ (**4**) and GM (**5**) with hydroxyl groups at C-7, compared to the activity of GA (**1**) and GB (**2**) lacking the 7-OH, showing that the 7-OH is not necessary for binding to PAFR, whereas hydroxyl groups at other positions appear to be less important. The study also confirmed that bilobalide (**6**), a terpene trilactone with only one five-membered carbocycle and three lactones, is not active in concentrations up to 100 μM (Table 1).

The seven photolabile analogs, GB derivatives **8a-c** and **11** and GC derivatives **9a-c**, with aromatic substituents at 10-OH all improved the affinity to the PAFR relative to the activities of GB (**2**) and GC (**3**) (Table 2). This is in agreement with previous SAR studies of GB (**2**) (Park, 1996; Hu, 1999; Hu, 2000), as well as a 3D-QSAR study on ginkgolides (Chen, 1998). However, it is interesting to note that aromatic substitutions at 10-OH of GC (**3**) as in compounds **9a-c**, improve the affinity to PAFR ca. 20 fold (Fig. 3*C*) thus making them equipotent to GB (**2**), while the same substitutions in GB (**2**) increases the affinity only 6-fold (Fig. 3B). Furthermore, the similar affinities of GB derivatives **8a-c** (Fig. 3*B*) and **11** and GC derivatives **9a-c** (Fig. 3*C*), respectively, implies that it is the steric bulk or the lipophilicity of the substituents, rather than the specific functional groups that are important for the increase in affinity (Table 2).

GC derivatives **10a** and **10b** (Figure 4) with dansyl groups at 7-OH and 1-OH are less potent and equipotent, respectively to their parent compound, 10-*O*-benzophenone-GC (**9a**) (Fig. 3*D*). In

compound **10a**, which is ca. 6 times less active than **9a** (Table 2), the bulk at the 7 position seems to be responsible for the reduction in affinity. The fact that compound **10b** is equipotent to **9a** suggests that once a bulky aromatic group occupies this area, further aromatic groups neither increase nor decrease the affinity.

The 7-fluoro GB (**13**, Figure 6) was essentially equipotent to GB (**2**), and ca. 10 times more potent than GC (**3**); thus, the [^{18}F]-labeled analog of **13** could be a useful probe for visualizing the PAFR binding sites in mammalian brain. Furthermore, **13** could be used for probing interactions of [^{18}F]-**13** with targets other than the PAFR. The substitution at C-7 of ginkgolides is critical for PAFR binding affinity; generally a β-OH group significantly decreases binding affinity, as in GC (**3**), GJ (**4**) and GM (**5**), while substitution with a dansyl group at 7-OH, as in **10a**, reduces this activity further. However, an α-fluorine at C-7 position has an affinity 10-fold higher than that of GC (**3**) and comparable to that of GB (**2**). The inverted stereochemistry at C-7, rather than electronic or steric effects of the fluorine substitution, may account for the enhanced activity.

Others who have made, or purported to make, Ginkgolide derivatives with modifications at C-7 did not appreciate the importance of stereochemistry at the C-7 position (Pietry, WO 99/52911; Ceazaux, GB 2,288,599; Vasella, US 6,143,725). The data presented shows the unexpected improvement in PAFR binding affinity when the C-7 substituent is an α- substituent. Thus, Ginkgolide derivatives having both an α- substituent at C-7 and a bulky or lipophillic substitution at 10-OH are expected to have yet further improved activity.

The two derivatives bearing alkyl substituents at C-10 of GB,

10-*O*-methyl GB (**14**) and 10-*O*-(2-fluoroethyl) GB (**15**), are both significantly less potent than GB (**2**). Thus, although the [^{11}C]-labeled derivatives are not suited for examination of interactions with the PAFR, both ligands should be useful for visualizing targets for ginkgolides in the brain, other than PAFR. Of course, all of the described compounds are expected to be useful for visualizing their targets in the brain, other than PAFR.

In conclusion, investigation of the effect of terpene trilactones isolated from *G. biloba* on the cloned PAFR have demonstrated that amongst the native compounds, GA (**1**) and GB (**2**) are the most potent. A series of photoactivatable analogs have been prepared, and PAFR binding assays showed that most of these analogs were more potent antagonists than their parent compounds, thus providing promising candidates for studies of the interaction of ginkgolides with the PAFR. The gingkolide derivative containing both a photoactivatable and a fluorescent group, compound **10b**, retained affinity to PAFR, and could therefore be useful in photolabeling and subsequent sequencing studies. Finally, the syntheses and assays of analogs that can be radiolabeled and used for PET studies have been described; in particular the radiolabeled derivative of 7-fluoro GB (**13**) could be a useful probe for *in vivo* PET studies of the PAFR, as well as potential new targets for ginkgolides.

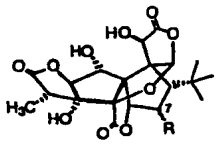
BIOLOGICAL TESTING - COMPOUNDS 16-31

Radioligand Binding Assay. The radioligand binding assays were performed as previously described (Shindou, 2000). In brief, membrane fractions from hearts and skeletal muscles of PAFR-Transgenic mice (50 µL suspension containing 158 fmol of PAFR) were mixed with 2 pmol of [^{3}H]-WEB 2086 in 50 µL of Buffer [25 mM Hepes/NaOH (pH 7.4), 0.25 M sucrose,10 mM $MgCl_2$, 0.1% BSA], and the compound to be tested in 100 µL of buffer in a 96-well microplate in triplicate for each concentration. These mixtures were incubated at 25°C for 90 min, upon which the receptor-bound [^{3}H]-WEB 2086 was filtered and washed with cold buffer. The filters were then dried at 50°C for at least 90 min., 25 µL of MicroScint-0 scintillation cocktail was added, and filters were placed in a TopCount microplate scintillation counter. Binding data were analyzed with the nonlinear curve-fitting program Microplate Manager III (Bio-Rad, Hercules, CA). Non-specific binding was determined using methods as previously described (Shindou, 2000).

RESULTS

Pharmacology. Derivatives **17-23** and **25-31** were tested for their ability to displace [^{3}H]-WEB 2086 binding to cloned PAFR (Tables 2 and 3) using membrane fractions from hearts and skeletal muscles of PAFR transgenic mice, as previously described (Shindou, 2000). In these fractions, GB (1) had a K_i value of 0.88 µM, thus being similar to the previously determined K_i value of 0.56 µM (Strømgaard, 2002)

Table 4. The K_i values of ginkgolide B (1) and C (2), and 7-epi derivatives

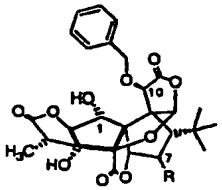


Compound	R	K_i (μM)[a]	Compound	R	K_i (μM)[a]
GB (1)	H	0.88	7α-F-GB (21)	α-F	0.99
GC (2)	β-OH	12.6[b]	7α-Cl-GB (22)	α-Cl	0.11
7α-OAc-GB (17)	α-OAc	7.84	7α-NHMe-GB (25)	α-NHMe	0.61
7-epi-GC (18)	α-OH	4.26	7α-NHEt-GB (26)	α-NHEt	1.62
7α-$OCOCH_2Ph$-GB (19)	α-$OCOCH_2Ph$	2.40	7α-NH_2-GB (27)	α-NH_2	8.64
7α-N_3-GB (20)	α-N_3	0.55			

[a]Inhibition of [^{3}H]-WEB 2086 binding. Values are means of two independent experiments performed in triplicate. [b]Data from previous studies (Strømgaard, K., *et al.*).

Derivatives with 7α-substituents were all more potent than GC (2) (Table 4), but within this group of compounds there were marked differences; 7α-OAc, 7α-OCOBn, 7α-OH and 7α-NH_2 ginkgolide B derivatives all had K_i values between 2.4-7.8 μM, thus being slightly more potent than GC (2), but still significantly less potent than GB (1). Compounds **20**, **21**, **25** and **26** with 7α-N_3, 7α-F, 7α-NHMe and 7α-NHEt substituents, respectively, were equipotent to GB (1) with K_i values in the range of 0.55-1.62 μM. Finally, 7α-chloro ginkgolide B (22) was the most potent compound in this series with a K_i value of 0.11 μM, thus being the most potent non-aromatic ginkgolide derivative described.

Table 5. The K_i values of benzylated derivatives.



Compound	R	K_i (μM)[a]	Compound	R	K_i (μM)[a]
10-OBn-GB (28)	H	0.12	10-OBn-7α-F-GB (30)	α-F	0.10
10-OBn-GC (29)	β-OH	1.67	10-OBn-epi-GC (31)	α-OH	0.60

[a]Inhibition of [^{3}H]-WEB 2086 binding. Values are means of two independent experiments performed in triplicate.

The relactonized compound **23** (Scheme 2) was also tested for binding to PAFR and was found to be essentially inactive with a K_i value > 40 μM. Benzyl derivatives were investigated as well (Table 3), and as expected a 10-*O*-benzyl group significantly improved the affinity for PAFR. Compounds **28** and **30** were the most potent with K_i values of 0.12 and 0.10 μM, respectively, while 10-*O*-benzyl-GC (**29**) and 10-*O*-benzyl-7-epi-GC (**31**) were slightly less potent with K_i values of 1.67 and 0.60 μM, respectively.

DISCUSSION

Structure-activity relationship (SAR) studies of ginkgolides on the PAFR have primarily focused on GB (**1**) (Figure 8) derivatives (Corey, 1989; Corey, 1991; Park, U.S. Patent No. 5,541,183; Hu, 1999; Hu, 2000) as outlined in Figure 8, e.g. the importance of lactones and the *tert*-butyl group has been investigated, whereas the effect of stereochemistry of hydroxyl groups remains to be examined. Ginkgolide C (GC, **2**, Figure 8), having a hydroxyl group at C-7, is significantly less potent than GB (**1**) (Strømgaard, 2002), which has been

explained by the hydrophilic 7β-OH of GC (2) being next to the lipophilic *tert*-butyl group, which is believed to interact with a lipophilic pocket in the PAFR (Braquet, 1991). Moreover, substitution at 7-OH further decreases antagonistic activity, as demonstrated by 7-*O*-(4-methylphenyl)-GB that was devoid of PAFR activity (Pietri, 2001), and a 7-*O*-dansyl-GB derivative was also less potent than the parent compound (Strømgaard, 2002).

Preliminary studies showed that 7α-F-GB was equipotent to GB, and 15-fold more potent as a PAFR antagonist as compared to GC (2) (Suehiro, M., *et al.*), despite the fact that fluorine is sterically equivalent to OH, and more polar than hydrogen (Smart, 2001). Since configuration of the fluorine atom is α, whereas that of the 7-hydroxyl in GC (2) is β, it is not clear whether this difference in activity is due to changes in stereochemistry, steric effects or electronic effects. In the following, we describe a series of ginkgolide derivatives with variation at the critical 7-position, and the assessment of these derivatives for their ability to displace radioligand binding to cloned PAFR.

Herein the effect of modification of the C-7 position of ginkgolides has been investigated by synthesis of fifteen analogs (17-31), which have been prepared from native ginkgolides GB (1) and GC (2), and evaluated with the cloned PAFR.

The derivatives with 7α-substituents were prepared by nucleophilic substitution of 7β-OTf-GB (16), but in several cases these reactions did not proceed as expected. Attempts to introduce larger halogens such as bromine and iodine, as well as other nucleophiles failed. In the reaction between 16

and $NaSCOCH_3$, the 7-OTf group remained intact; instead the reaction presumably took place at C-11 of lactone C to give **24** (Scheme 2). Reaction of **16** with MeOH and 2,6-lutidine gave rise to neoginkgolide C (**23**) with a novel rearranged skeleton (Scheme 2). This compound had a K_i value > 40 μM, which is in agreement with previous studies which showed that modification of lactone C significantly reduced PAFR binding (Figure 8) (Hu, 2001).

During the reduction of azide **20** interesting observations were made; when carried out in MeOH this reaction did not give the expected primary amine **27**, but gave *N*-methylamine **25** instead, and when carried out in EtOH the reduction gave *N*-ethylamine **26**. Besides being a potential novel procedure for a direct conversion of azides into alkylated amines, it also raises mechanistic considerations. Treatment of **27** with Pd/C in MeOH gave **25**, albeit in lower yield than starting from azide **20**, and with several side products. This implies that in the preparation of **25** and **26** the azide **20** is initially reduced to amine **27**, which then reacts instantly with the oxidized solvent to form an imine, that is reduced to yield the products. Further studies should confirm this pathway, as well as the generality of this reaction.

The prepared derivatives were tested for binding to cloned PAFR (Tables 4 and 5). It was observed that 7α-derivatives were slightly more potent than the 7β-derivatives, as GC (**2**) had a K_i value of 12.6 μM, while for 7-epi-GC (**18**) K_i is 4.26 μM. Likewise 7α-OAc-GB (**17**) had a K_i of 7.84 μM, while 7β-OAc-GB (i.e., 7-OAc-GC) had been shown to have low potency comparable to that of GC (**2**) (Jaracz, 2002). Furthermore, the 7β-derivative **19** is a reasonably potent PAFR antagonist with a K_i value of 2.40 μM (Table 4) in contrast to 7β-*O*-(4-

methylphenyl)-GB, which is devoid of PAFR activity (Pietri, 2001).

The nature of the 7α-substituent, on the other hand, had a major impact on the binding to PAFR. It appears that polarizable substituents at C-7 lead to increased potency. Introduction of azide and fluorine groups yielded compounds that were equipotent to GB (**1**) (Table 4), while introduction of a chlorine as in 7α-Cl-GB (**22**) leads to a dramatic increase in binding affinity. Thus **22** with K_i = 0.11 μM, was 115-fold more potent than GC (**2**), and 8-times more potent than GB (**1**), thereby being the most potent non-aromatic ginkgolide derivative described to date. On the other hand, it appears that polar groups that can form hydrogen bonds decrease activity, as seen in the case of a hydroxyl group at C-7 to give 7-epi-GC (**18**) and an amino group to give 7α-NH_2-GB (**27**), compounds with binding affinities lower than GB. However, alkylation of **27** to give 7α-NHMe-GB (**25**) and 7α-NHEt-GB (**26**) led to significant increases in binding affinities, with K_i values of 0.61 μM and 1.62 μM, respectively. It may be that such alkylations of the 7α-amino group sterically disfavors hydrogen bonding. Nevertheless, rationalization of these trends requires further developments in ongoing molecular mechanistic studies of the ginkgolide / PAFR interaction.

Introduction of benzyl groups in the 10-OH position of ginkgolides is known to improve affinity for PAFR (Park, P.-U., *et al.*, U.S. Patent No. 5,541,183; Hu, 2000; Strømgaard, 2002), but whether this is true for 7α-substituted derivatives was not known. The affinities of 10-*O*-benzyl-7α-F-GB (**30**) and 10-*O*-benzyl-7-epi-GC (**31**) (Table 5) shows 10- and 7-fold improved affinity compared to their non-benzylated

derivatives. Thus 10-benzylation of 7α-substituted derivatives improves binding affinity as previously shown for other ginkgolide derivatives.

In conclusion we have synthesized several ginkgolide derivatives with modifications at C-7. These syntheses have led to several unexpected products such as **23** and **24**, as well as a potential novel procedure for a direct conversion of azides into alkylamines. Moreover, contrary to previous convictions, we have shown that introducing lipophilic groups in the C-7 position, in particular chlorine, significantly improves PAFR affinity compared to GB (**1**). This gives rise to new possibilities for improving affinity of ginkgolides to PAFR, as well as providing material for future SAR studies of ginkgolides and PAFR.

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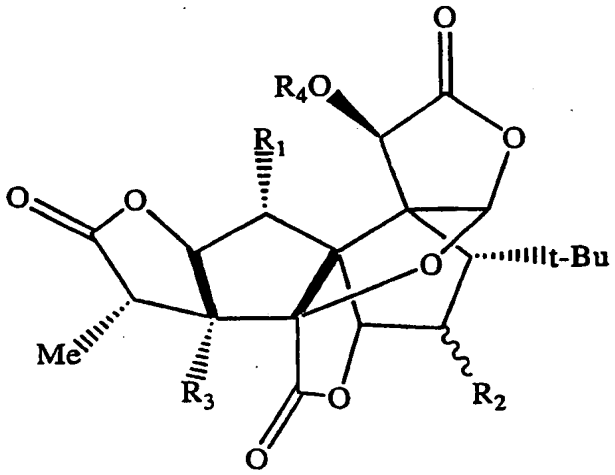
http://www.herbs.org/greenpapers/ginkgo.htm

What is claimed is:

1. A compound having the structure:



wherein R_1 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

wherein R_2 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety;

wherein R_3 is H or OH;

wherein R_4 is H, OH, a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety; and

wherein at least one of R_1, R_2, R_3, or R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety,

or an optically pure enantiomer of the compound.

2. The compound of claim 1, wherein R_1 is a fluorescent moiety and each of R_2 and R_4 is H or OH.

3. The compound of claim 1, wherein R_2 is a fluorescent moiety or a radioactive moiety and each of R_1 and R_4 is H or OH.

4. The compound of claim 1, wherein R_4 is a photoactivatable

moiety or a radioactive moiety and each of R_1 and R_2 is H or OH.

5. The compound of claim 1, wherein the photoactivatable moiety is a phenylazide, a purine or pyrimidine azides, a diazoacetate, a diazoketone, a nitrobenzene, or an aryldiazonium salt.

6. The compound of claim 1, wherein the photoactivatable moiety is benzophenone, trifluoromethyldiazirine tetrafluorophenyl, 8-azidoadenosine, 2-azidoadenosine, or 3H,3-aryldiazirine.

7. The compound of claim 1, wherein the fluorescent moiety is a fluorescent amine.

8. The compound of claim 1, wherein the fluorescent moiety is 5-(dimemethylamino)naphthalene-sulfonyl (dansyl), 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, or 4-(9-Anthroyloxy)phenacyl bromide.

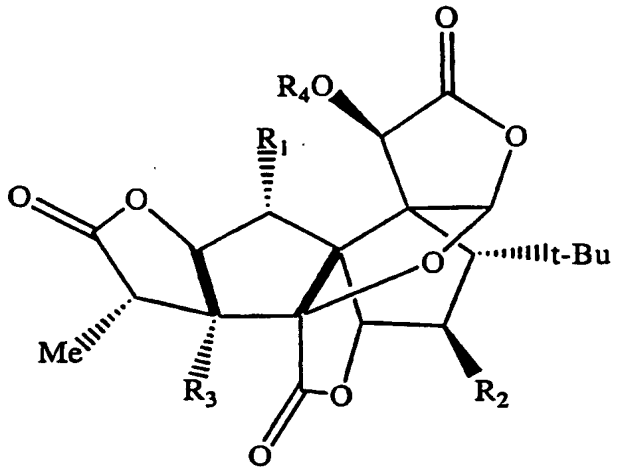
9. The compound of claim 1, wherein the radioactive moiety is ^{11}C, ^{13}N, ^{15}O, ^{3}H or ^{18}F.

10. The compound of claim 6, wherein the photoactivatable moiety is benzophenone, trifluoromethyldiazirine or tetrafluorophenyl.

11. The compound of claim 8, wherein the fluorescent moiety is 5-(dimemethylamino)naphthalene-sulfonyl.

12. The compound of claim 9, wherein the radioactive moiety is ^{18}F, ^{11}C or ^{3}H.

13. The compound of claim 1 having the structure:



14. The compound of claim 13, wherein R_1 is -O-5-(dimemethylamino)naphthalene-sulfonyl.

15. The compound of claim 13, wherein R_2 is -O-5-(dimemethylamino)naphthalene-sulfonyl.

16. The compound of claim 13, wherein R_2 is $-^{11}CH_3$.

17. The compound of claim 13, wherein R_2 is $-CH_2CH_2{}^{18}F$.

18. The compound of claim 1, wherein R_2 is ^{18}F.

19. The compound of claim 13, wherein R_2 is ^{3}H.

20. The compound of claim 13, wherein R_4 is a benzophenone moiety.

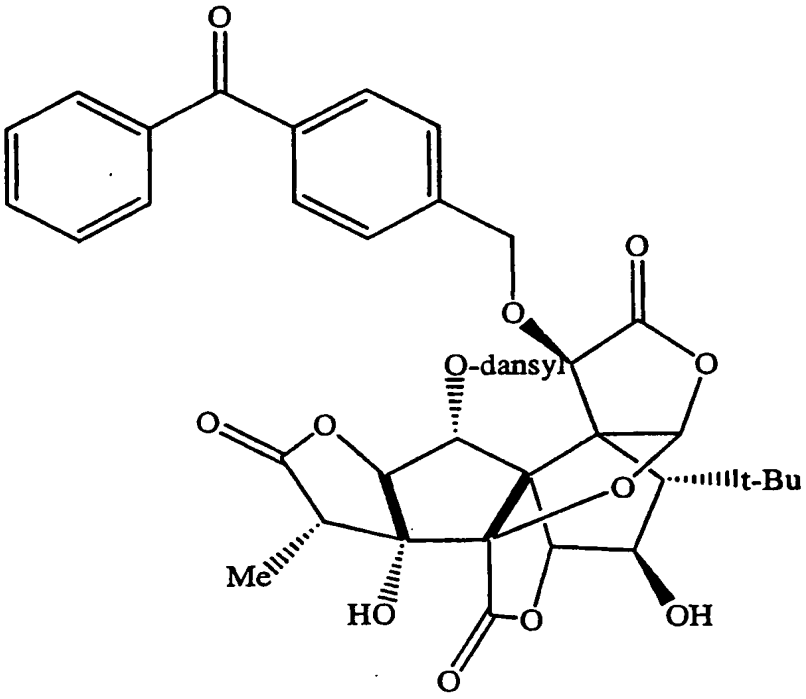
21. The compound of claim 13, wherein R_4 is a trifluoromethyldiazirine moiety.

22. The compound of claim 13, wherein R_4 is a tetrafluorophenyl azide moiety.

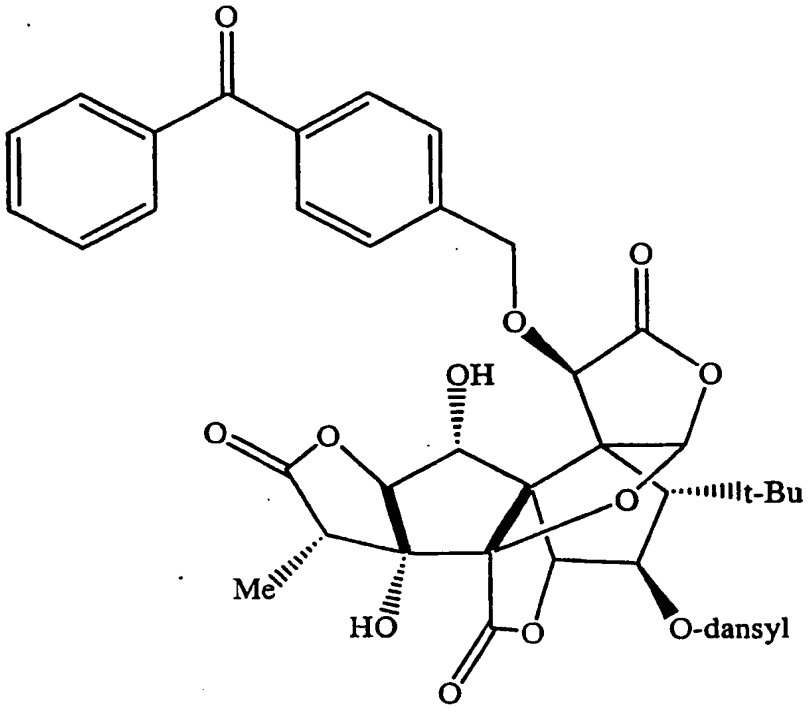
23. The compound of claim 13, wherein R_4 is $-^{11}CH_3$.

24. The compound of claim 13, wherein R_4 is $-CH_2CH_2{}^{18}F$.

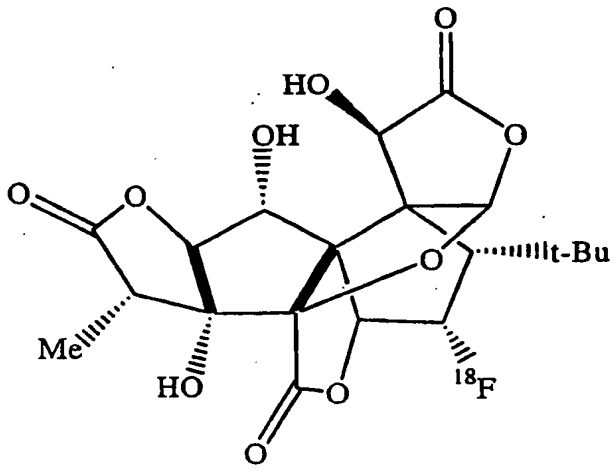
25. The compound of claim 14, having the structure:



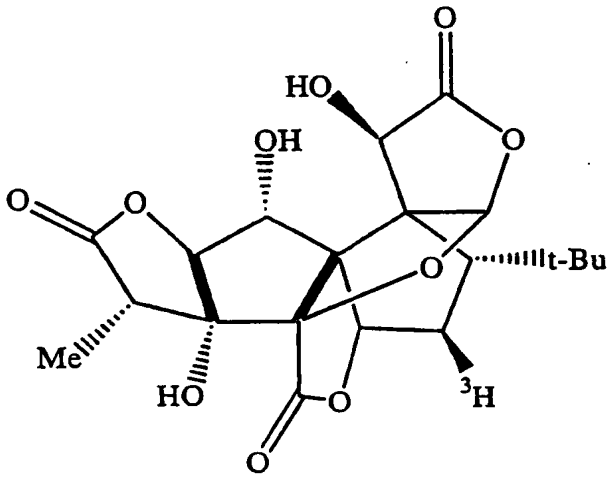
26. The compound of claim 15 having the structure:



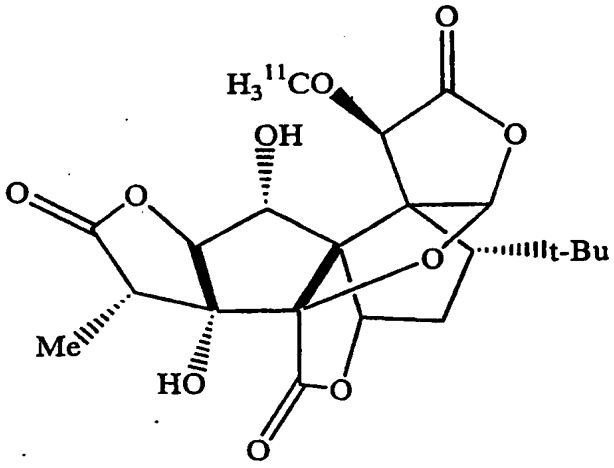
27. The compound of claim 18 having the structure:



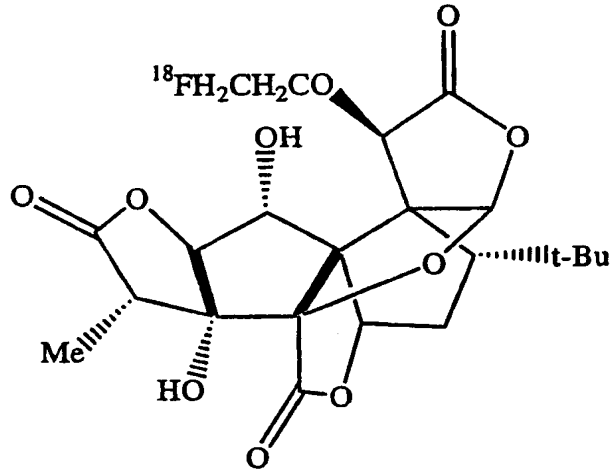
28. The compound of claim 19 having the structure:



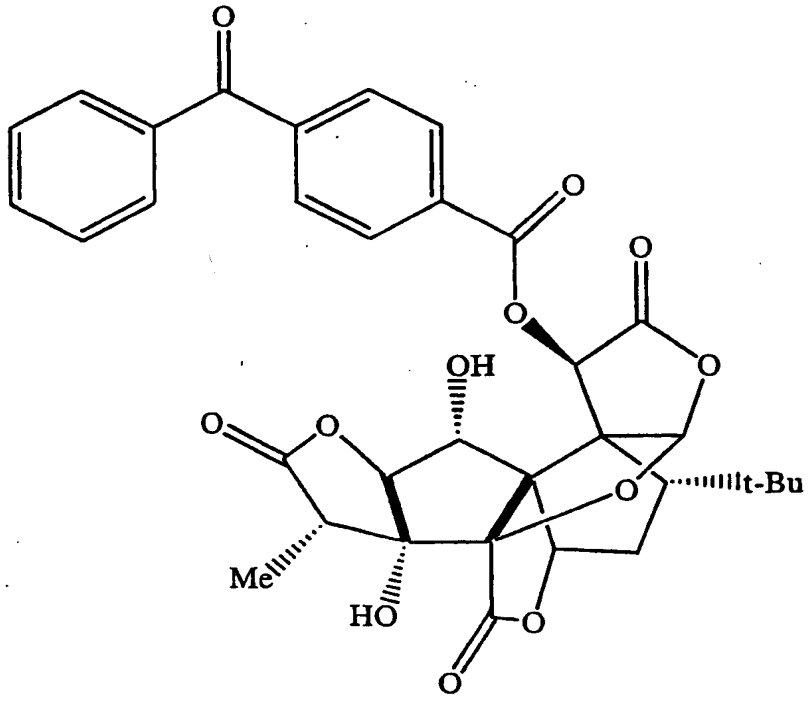
29. The compound of claim 23 having the structure:



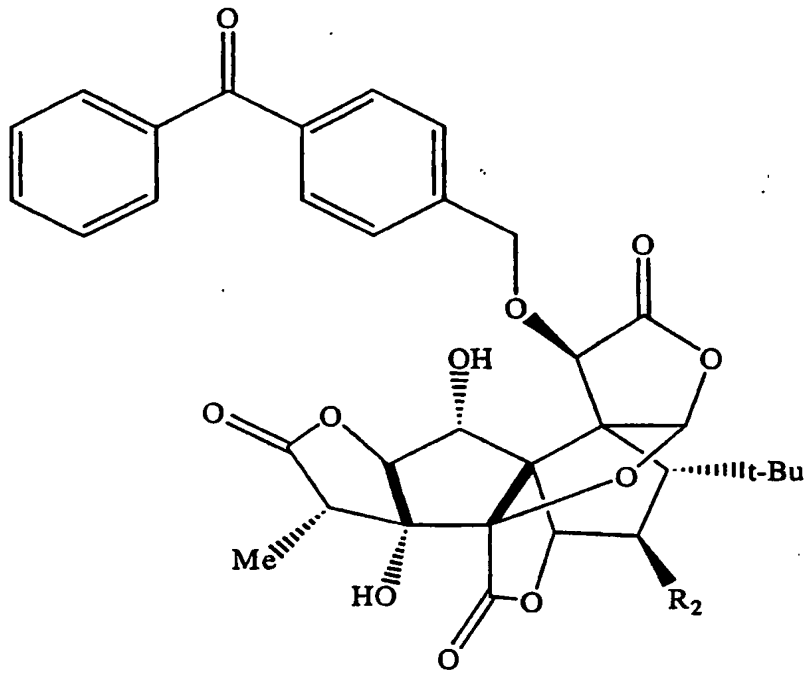
30. The compound of claim 24 having the structure:



31. The compound of claim 13 having the structure:

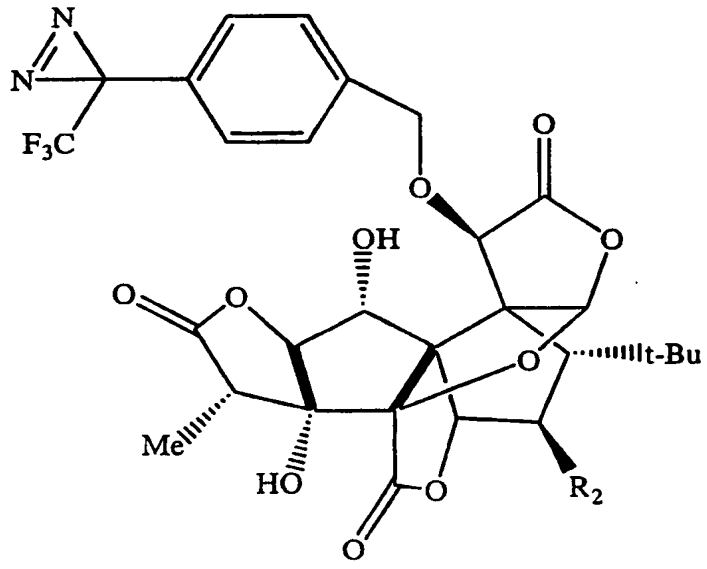


32. The compound of claim 20 having the structure:



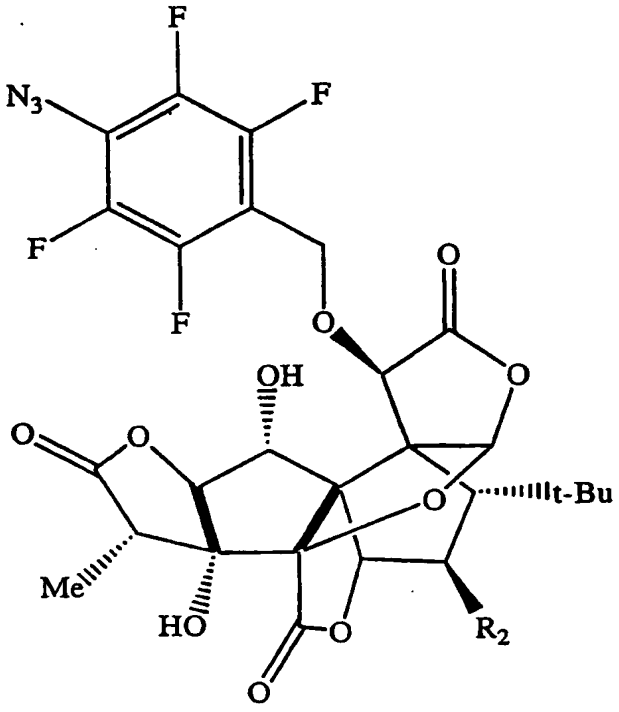
wherein R_2 is H or OH.

33. The compound of claim 21 having the structure:



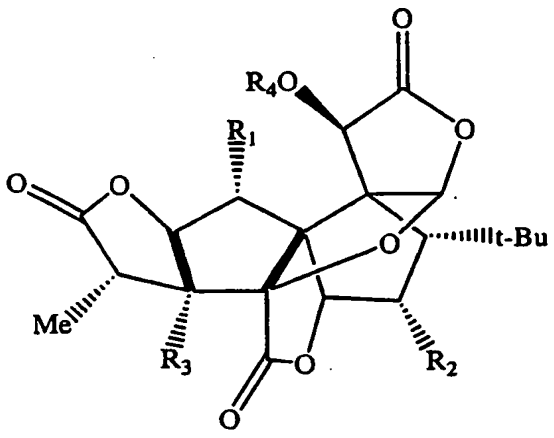
wherein R_2 is H or OH.

34. The compound of claim 22 having the structure:



wherein R_2 is H or OH.

35. A compound having the structure:



wherein R_1 is H or OH,

wherein R_2 is H, OH, halogen, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, $-N_3$, $-COR_5$, $-CONR_5R_6$, $-CO_2R_5$, $-OCOR_5$, $-NH(OH)$, $-NR_5R_6$, $-NHCOR_5$, $-N(OH)COR_5$, $-CH_2OR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, or $-NR_5SO_2R_6$,

where R_5 and R_6 are each, independently, hydrogen, substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms;

wherein R_3 is H or OH; and

wherein R_4 is H, OH, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_6, or $-R_6$,

where A is (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8)alkynyl, which is unsubstituted or substituted by a straight or branched alkyl chain group having 1 to 5 carbon atoms;

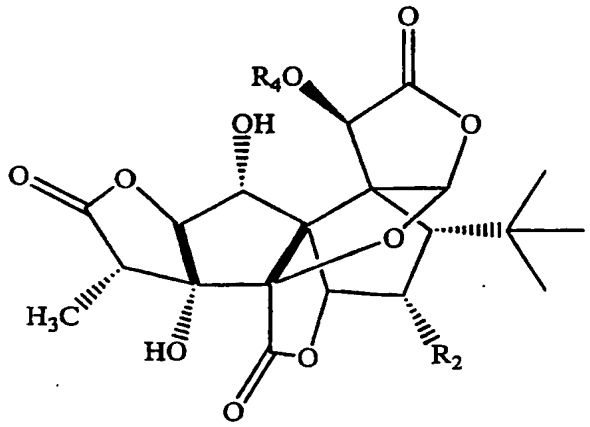
Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, substituted or unsubstituted (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen;

or an optically pure enantiomer, or a tautomer, or a salt of the compound.

36. The compound of claim 35 having the structure:



wherein R_2 is Cl, F, OH, a substituted or unsubstituted, straight or branched (C_1-C_5) alkyl, (C_2-C_5) alkenyl, (C_2-C_5) alkynyl, (C_1-C_5) alkoxy, (C_2-C_5) alkenyloxy, or (C_2-C_5) alkynyloxy, $-N_3$, $-COR_5$, $-CONR_5R_6$, $-CO_2R_5$, $-OCOR_5$, $-NH(OH)$, $-NR_5R_6$, $-NHCOR_5$, $-N(OH)COR_5$, $-CH_2OR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, or $-NR_5SO_2R_6$,

where R_5 and R_6 are each, independently, hydrogen, substituted or unsubstituted (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl, or a cycloalkyl or aryl group having 3 to 10 carbon atoms;

wherein R_4 is H or R_8;
wherein R_4 is R_8 when R_2 is F;

wherein R_8 is (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or $-A-NR_6$,

where A is an unsubstituted, straight chain (C_1-C_5)alkyl, (C_2-C_5) alkenyl, (C_2-C_5) alkynyl;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, hydroxy, substituted or unsubstituted (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, phenyl, phenoxy, aralkyl, or aralkyloxy, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, or $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, -CN, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a tautomer, or a salt

of the compound.

37. The compound of claim 36, wherein R_4 is H or -A-Ar.

38. The compound of claim 37, wherein R_4 is H or $-CH_2C_6H_5$.

39. The compound of claim 36, wherein R_2 is OH, Cl, $-N_3$, $-OCOR_3$, or $-NR_3R_4$.

40. The compound of claim 36, wherein R_2 is OH, Cl, $-OCOCH_3$, $-OCOCH_2C_6H_5$, $-N_3$, $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$.

41. The compound of claim 36, wherein R_2 is OH, Cl, $-OCOCH_3$, $-OCOCH_2C_6H_5$, $-N_3$, $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$; and wherein R_4 is H or $-CH_2C_6H_5$.

42. The compound of claim 35, wherein R_1 is OH, R_2 is F, R_3 is OH, and R_4 is H.

43. The compound of claim 36, wherein R_2 is Cl, and R_4 is H.

44. The compound of claim 36, wherein R_2 is $-N_3$, and wherein R_4 is H.

45. The compound of claim 36, wherein R_2 is $-NHCH_3$, and wherein R_4 is H.

46. The compound of claim 36, wherein R_2 is $-NHCH_2CH_3$, and wherein R_4 is H.

47. The compound of claim 36, wherein R_2 is $-OCOCH_2C_6H_5$, and wherein R_4 is H.

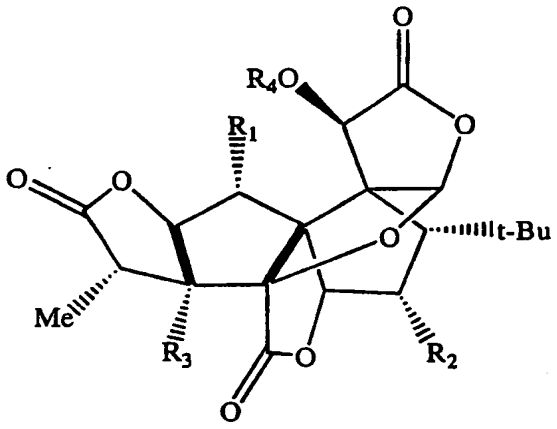
48. The compound of claim 36, wherein R_2 is F, and wherein R_4 is $-CH_2C_6H_5$.

49. The compound of claim 36, wherein R_2 is Cl, and wherein R_4 is $-CH_2C_6H_5$.

50. The compound of claim 36, wherein R_2 is H, and wherein R_4 is $-CH_2C_6H_5$.

51. The compound of claim 36, wherein R_2 is OH, and wherein R_4 is $-CH_2C_6H_5$.

52. The compound of claim 35 having the structure:



wherein R_1 is H or OH;

wherein R_2 is H, F, Br, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl group, (C_2-C_5) alkenyl, or a (C_2-C_5) alkynyl;

wherein R_3 is H or OH; and

wherein R_4 is H, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_6, or $-R_6$, where A is an alkylene group having 1 to 8 carbon atoms, which is unsubstituted or substituted by a straight or branched alkyl chain group having 1 to 5 carbon atoms;

Z is carbon, oxygen, sulfur or nitrogen;

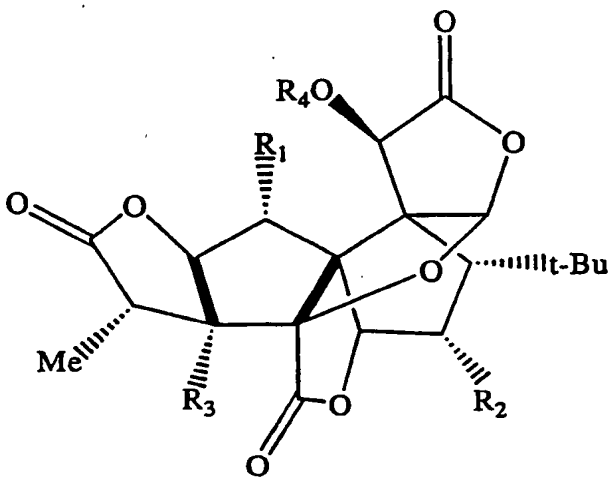
Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, a (C_1-C_{10}) haloalkyl, an (C_1-C_{10}) alkoxy, an (C_2-C_{10}) alkenyloxy, an (C_2-C_{10}) alkynyloxy, a (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_6$, $-CONR_6R_6$, $-CO_2R_6$, $-NHCOR_6$, $-NH(OH)$, $-N(OH)COR_6$, $-CH_2OR_6$, $-OCH_2CO_2R_6$, $-CH_2SR_6$, $-CH_2NR_6R_6$, $-SR_6$, $-OSR_6$, $-NR_6R_6$, $-NR_6SO_2R_6$,

where R_6 is hydrogen, (C_1-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

53. The compound of claim 52, wherein R_2 is F.

54. The compound of claim 35, having the structure:



wherein R_1 is H, or OH;

wherein R_2 is H, OH, F, Br, unsubstituted or substituted, straight or branched (C_1-C_5) alkyl, (C_2-C_5) alkenyl, or (C_2-C_5) alkynyl;

wherein R_3 is H or OH; and

wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_5, or -R_6,

where A is an (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a phenyl group, a phenoxy group, an aralkyl group, an

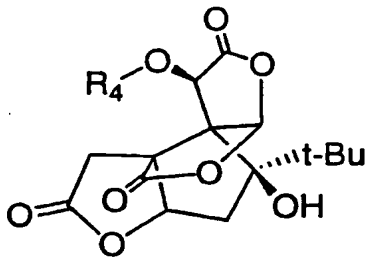
aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$, $-NHCOR_5$, $-NH(OH)$, $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, (C_1-C_{10}) alkyl or a (C_3-C_{10}) cycloalkyl, $-SCX_3$ in which X is a halogen, -CN, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

55. The compound of claim 54, wherein R_2 is F.

56. A compound having the structure:



wherein R_4 is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety.

57. The compound of claim 56, wherein the photoactivatable moiety is a phenylazide, a purine or pyrimidine azides, a diazoacetate, a diazoketone, a nitrobenzene, or an aryldiazonium salt.

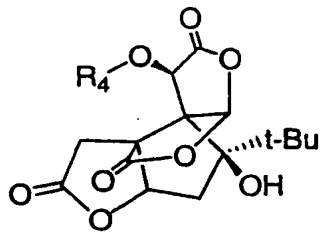
58. The compound of claim 56, wherein the photoactivatable moiety is benzophenone, trifluoromethyldiazirine tetrafluorophenyl, 8-azidoadenosine, 2-azidoadenosine, or 3H,3-aryldiazirine.

59. The compound of claim 56, wherein the fluorescent moiety is a fluorescent amine.

60. The compound of claim 56, wherein the fluorescent moiety is 5-(dimemethylamino)naphthalene-sulfonyl (dansyl), 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, or 4-(9-Anthroyloxy)phenacyl bromide.

61. The compound of claim 55, wherein the radioactive moiety is ^{18}F, ^{11}C, ^{3}H.

62. A compound having the structure:



wherein R_4 is H, OH, -A-Ar, -A-Z-Ar, $-SO_2$-Ar, or -A-NR_5, or $-R_6$,

where A is a (C_1-C_8) alkyl group, which is unsubstituted or substituted by a straight or branched (C_1-C_5) alkyl chain;

Z is carbon, oxygen, sulfur or nitrogen;

Ar is a phenyl group, a pyridyl group, a naphthyl group, a pyrimidyl group, or a quinolyl group, each of which may contain heteroatoms and may be unsubstituted or substituted by one to five substituents selected from the group consisting of hydrogen, halogen, a hydroxy group, a carboxylic acid group, (C_1-C_{10}) alkyl, (C_2-C_{10}) alkenyl, (C_2-C_{10}) alkynyl, (C_1-C_{10}) haloalkyl, (C_1-C_{10}) alkoxy, (C_2-C_{10}) alkenyloxy, (C_2-C_{10}) alkynyloxy, (C_1-C_{10}) haloalkoxy, a

phenyl group, a phenoxy group, an aralkyl group, an aralkyloxy group, a substituted phenyl group, a substituted phenoxy group, a substituted aralkyl group, a substituted aralkyloxy group, $-COR_5$, $-COR_6$, $-CONR_5R_6$, $-CO_2R_5$, $-NHCOR_5$, $-NH(OH)$, $-N(OH)COR_5$, $-CHOR_5$, $-OCH_2CO_2R_5$, $-CH_2SR_5$, $-CH_2NR_5R_6$, $-SR_5$, $-OSR_5$, $-O_2NR_5R_6$, $-NR_5R_6$, $-NR_5SO_2R_6$,

in which R_5 and R_6 are the same or different and each is hydrogen, (C_1-C_{10}) alkyl or a (C_3-C_{10}) cycloalkyl group, $-SCX_3$ in which X is a halogen, $-CN$, $-NO_2$ or -Z-A-Z'- in which Z and A are as defined above and Z' represents carbon, oxygen, sulfur, or nitrogen,

or an optically pure enantiomer, or a salt of the compound.

63. The compound of claim 35, 36, 52, 54, 55 or 62, wherein if any group is substituted, the substituent is halogen, hydroxyl, straight chain (C_1-C_5)alkyl, branched chain (C_3-C_5)alkyl, (C_3-C_{10})cycloalkyl, straight chain(C_1-C_5)alkylcarbonyloxy, branched chain (C_3-C_5)alkylcarbonyloxy, arylcarbonyloxy, straight chain(C_1-C_5)alkoxycarbonyloxy, branched chain(C_3-C_5)alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, straight chain(C_1-C_5)alkylcarbonyl, branched chain (C_3-C_5)alkylcarbonyl, straight chain (C_1-C_5)alkoxycarbonyl, branched chain (C_3-C_5)alkoxycarbonyl, aminocarbonyl, straight chain (C_1-C_5)alkylthiocarbonyl, branched chain (C_3-C_5)alkylthiocarbonyl, straight chain (C_1-C_5)alkoxyl, branched chain (C_1-C_5)alkoxyl, phosphate, phosphonate, cyano, amino, straight chain (C_1-C_5)alkylamino, branched chain (C_3-C_5)alkylamino, straight chain (C_1-C_5)dialkylamino, branched chain (C_3-C_5)dialkylamino, arylamino, diarylamino, straight chain (C_1-C_5)alkylarylamino, branched chain (C_3-C_5)alkylarylamino, acylamino, straight chain (C_1-C_5)alkylcarbonylamino, branched chain (C_3-C_5)alkylcarbonylamino, arylcarbonylamino, carbamoyl, ureido, amidino, imino, sulfhydryl, straight chain (C_1-C_5)alkylthio, branched chain (C_3-C_5)alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, azido, 4-10 membered heterocyclyl, straight chain (C_1-C_{30})alkylaryl, branched chain (C_3-C_{30})alkylaryl, or an aromatic or 5-6 membered heteroaromatic moiety,
which substituent may be further substituted by any of the above.

64. The pharmaceutically acceptable salt of the compound of any one of claims 35-55, wherein the salt is the chloride, mesylate, maleate, fumarate, tartarate, hydrochloride, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate and sulfate salts.

65. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any one of claims 35-55, 62 or 64, and a pharmaceutically acceptable carrier.

66. A process for the manufacture of a pharmaceutical composition comprising admixing the compound of any one of claims 35-55, 62 or 64 with a pharmaceutically acceptable carrier.

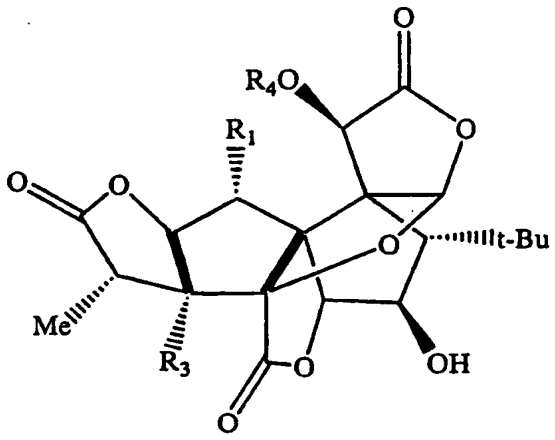
67. A method of inhibiting activation of the platelet-activating factor receptor (PAFR) which comprises contacting the PAFR with a compound of any one of claims 35-55, 62 or 64 so as to thereby inhibit activation of the PAFR.

68. A method of treating a platelet-activating factor (PAF) associated condition in a subject comprising administering to the subject an amount of the compound of any one of claims 35-55, 62 or 64 effective to treat the PAF-associated disease.

69. The method of claim 68, wherein the PAF-associated condition is a neurodegenerative disease, Alzheimer's disease, senile dementia, stroke, nerve cell damage due to ischemia, asthma, abnormal blood clot formation, endotoxic shock, myocardial ischemia or hyperacute rejection arising from post-renal transplant or xenoperfusion.

70. A process of preparing the compound of claim 35 comprising the steps of:

i) reacting a compound having the structure



wherein R_1, R_3 and R_4 are as defined in claim 35,

with trifluoromethanesulfonic anhydride under inert conditions to form a triflate; and

ii) reacting the triflate of step i) with a nucleophilic reagent in a polar-aprotic solvent to substitute the triflate with the nucleophile and to form the compound.

71. The process of claim 70, wherein the nucleophilic reagent is sodium acetate, sodium phenylacetate, sodium azide, tetrabutylammonium fluoride hydrate, or tetrabutylammonium chloride.

72. The process of claim 70, wherein the polar-aprotic solvent is dichloromethane, pyridine, dimethylformamide, methylsulfoxide, or acetonitrile.

73. The process of claim 71, wherein the nucleophilic reagent is sodium acetate or sodium phenylacetate, further comprising hydrolyzing the product of step ii) in the presence of 1N hydrochloric acid.

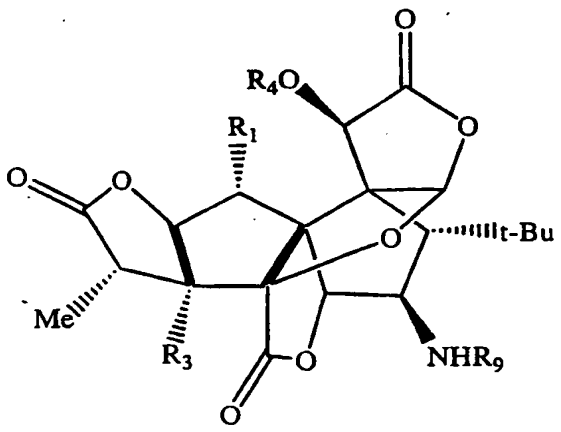
74. The process of claim 71, wherein the nucleophilic reagent is sodium azide, further comprising reacting the product of step ii) with hydrogen in the presence of palladium and carbon in methanol or ethanol.

75. The process of claim 71, wherein the nucleophile is tetrabutylammonium fluoride hydrate, further comprising reacting the product of step ii) with benzyl chloride.

76. A process of forming a secondary amine compound from an azide of the compound by contacting the azide of the compound with hydrogen, palladium and carbon in a polar-protic solvent.

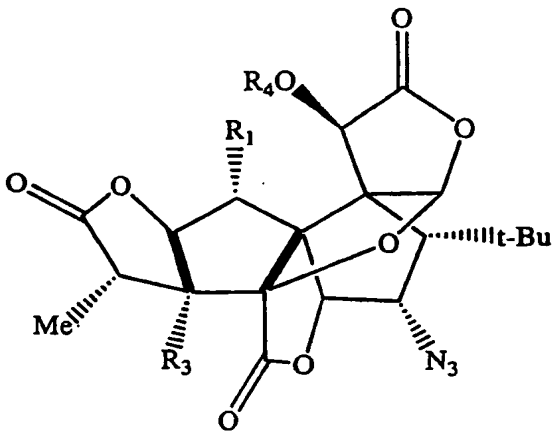
77. The process of claim 76, wherein the polar-protic solvent is an alcohol.

78. The process of claim 76, wherein the secondary amine compound has the structure:



wherein R_1, R_3 and R_4 are as defined in claim 1 and R_9 is an alkyl group,

and wherein the azide of the compound has the structure:



79. The process of claim 78, wherein the polar-protic solvent is an alcohol.

80. The process of claim 79, wherein the alcohol is CH_3OH and R_9 is CH_3.

81. The process of claim 79, wherein the alcohol is CH_3CH_2OH and R_9 is CH_3CH_2.

82. A process for detecting the binding of the compound of claim 1 to a target, comprising contacting the compound with the target and detecting the binding of the compound to the target.

83. The process of claim 82, wherein the target is a DNA.

84. The process of claim 82, wherein the target is a receptor.

85. The process of claim 82, wherein the target is an enzyme.

86. A process for detecting the localization of a receptor in a subject comprising administering the compound of claim 1 to the subject and detecting at any location in the subject's body to identify a point of accumulation of the compound so as to thereby localize the receptor in the subject, wherein localization of a receptor means a higher concentration of that receptor then at other points in the subject's body.

87. A process of identifying a target that binds the compound of claim 1, comprising contacting the compound with the target, isolating the target so as to thereby identify the target.

88. The process of claim 87, wherein the target is a DNA.

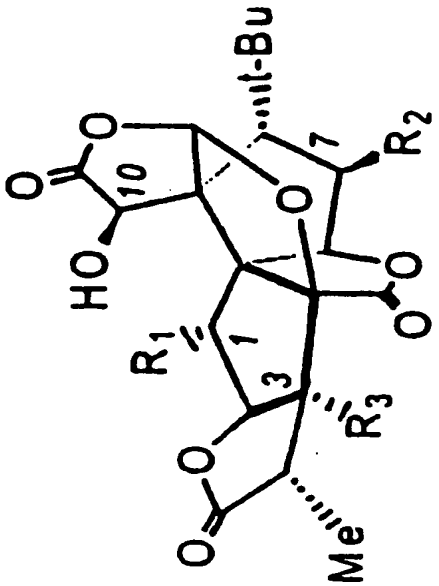
89. The process of claim 87, wherein the target is a receptor.

90. The process of claim 87, wherein the target is an enzyme.

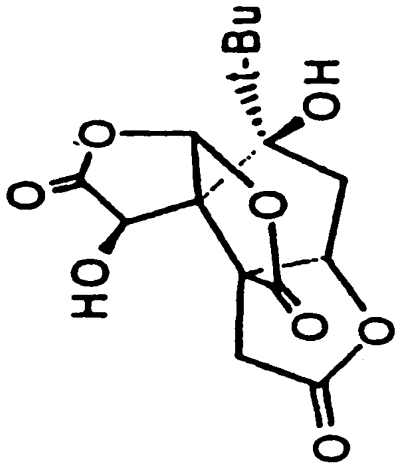
91. A process of identifying a receptor that binds the compound of claim 1 in a subject, comprising administering the compound to the subject, imaging the subject's body to identify the point of accumulation of the compound in the

subject, and identifying the receptor present at the point of accumulation of the compound, so as to thereby identify the receptor in the subject.

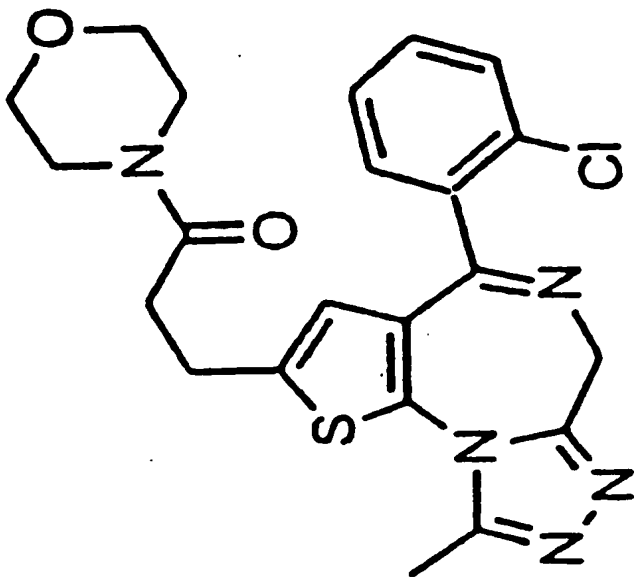
FIGURE 1



	R_1	R_2	R_3
Ginkgolide A (GA 1)	H	H	OH
Ginkgolide B (GB, 2)	OH	H	OH
Ginkgolide C (GC,3)	OH	OH	OH
Ginkgolide J (GJ, 4)	H	OH	OH
Ginkgolide M (GM, 5)	OH	OH	H



Bilobalide (BB, 6)



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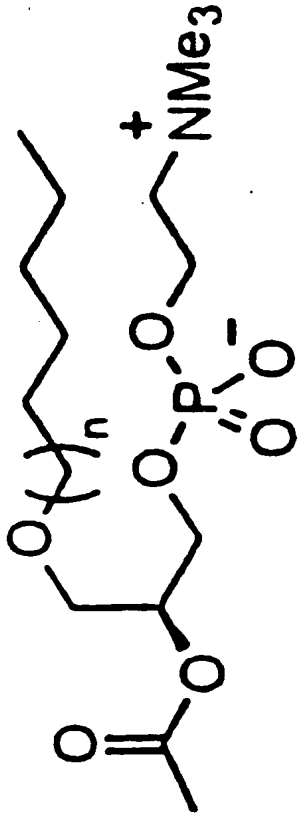


PAF (n = 11-13)

FIGURE 2

FIGURE 3

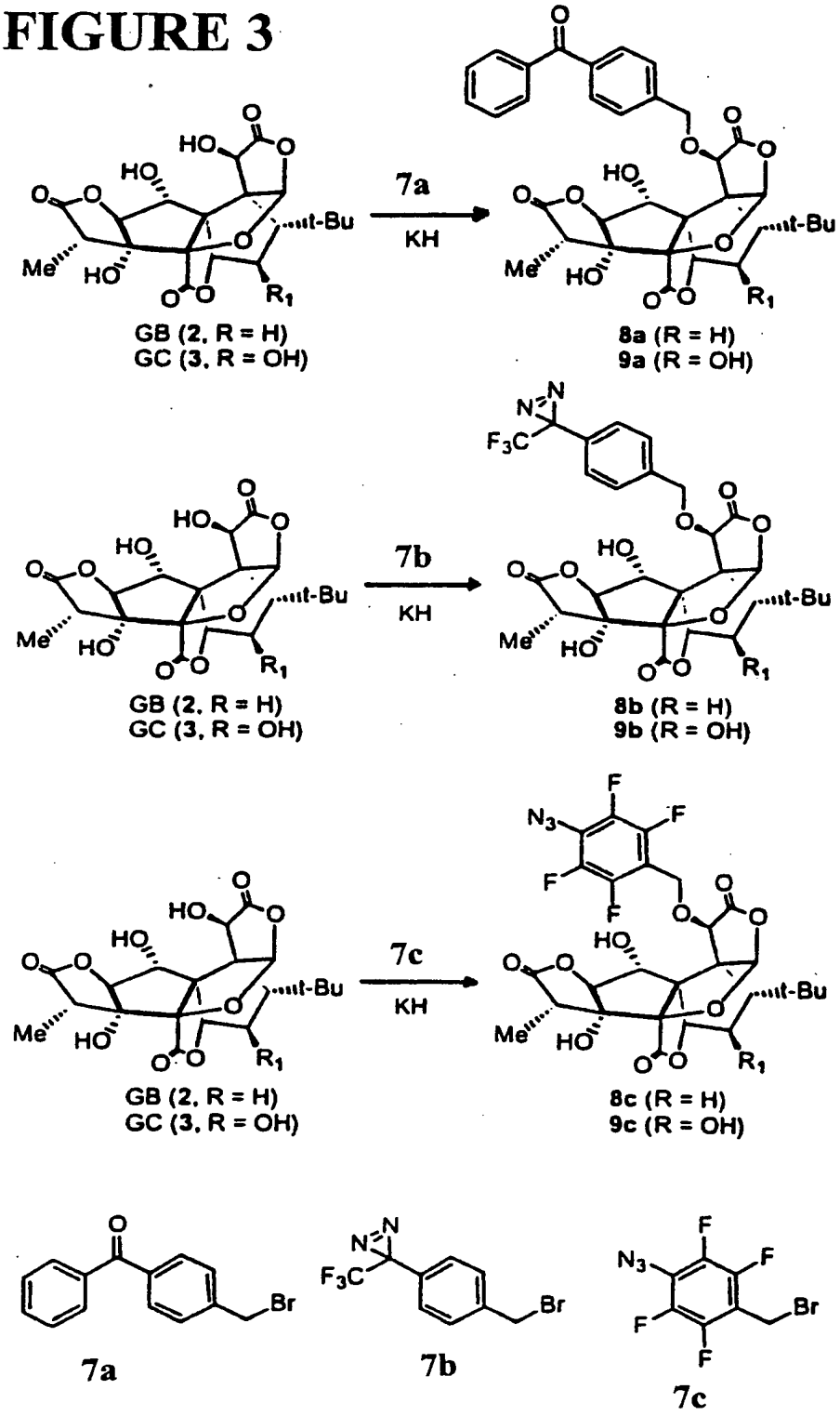

7a
KH
GB (2, R = H)
GC (3, R = OH)
8a (R = H)
9a (R = OH)
7b
KH
GB (2, R = H)
GC (3, R = OH)
8b (R = H)
9b (R = OH)
7c
KH
GB (2, R = H)
GC (3, R = OH)
8c (R = H)
9c (R = OH)
7a
7b
7c

FIGURE 4

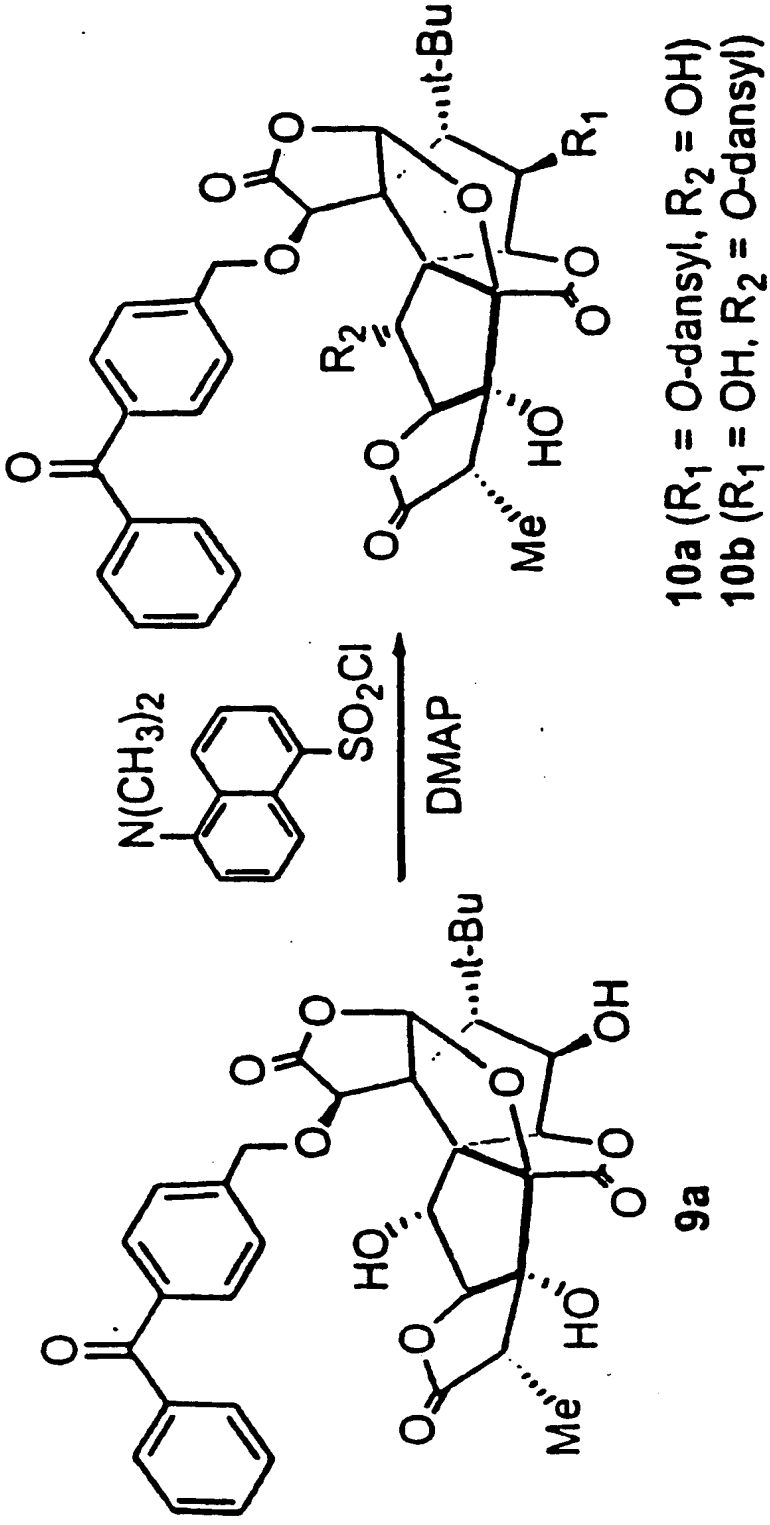

t-Bu
OH
HO
HO
Me
9a
N(CH3)2
SO2Cl
DMAP
t-Bu
R1
R2
HO
Me
10a (R1 = O-dansyl, R2 = OH)
10b (R1 = OH, R2 = O-dansyl)

FIGURE 5

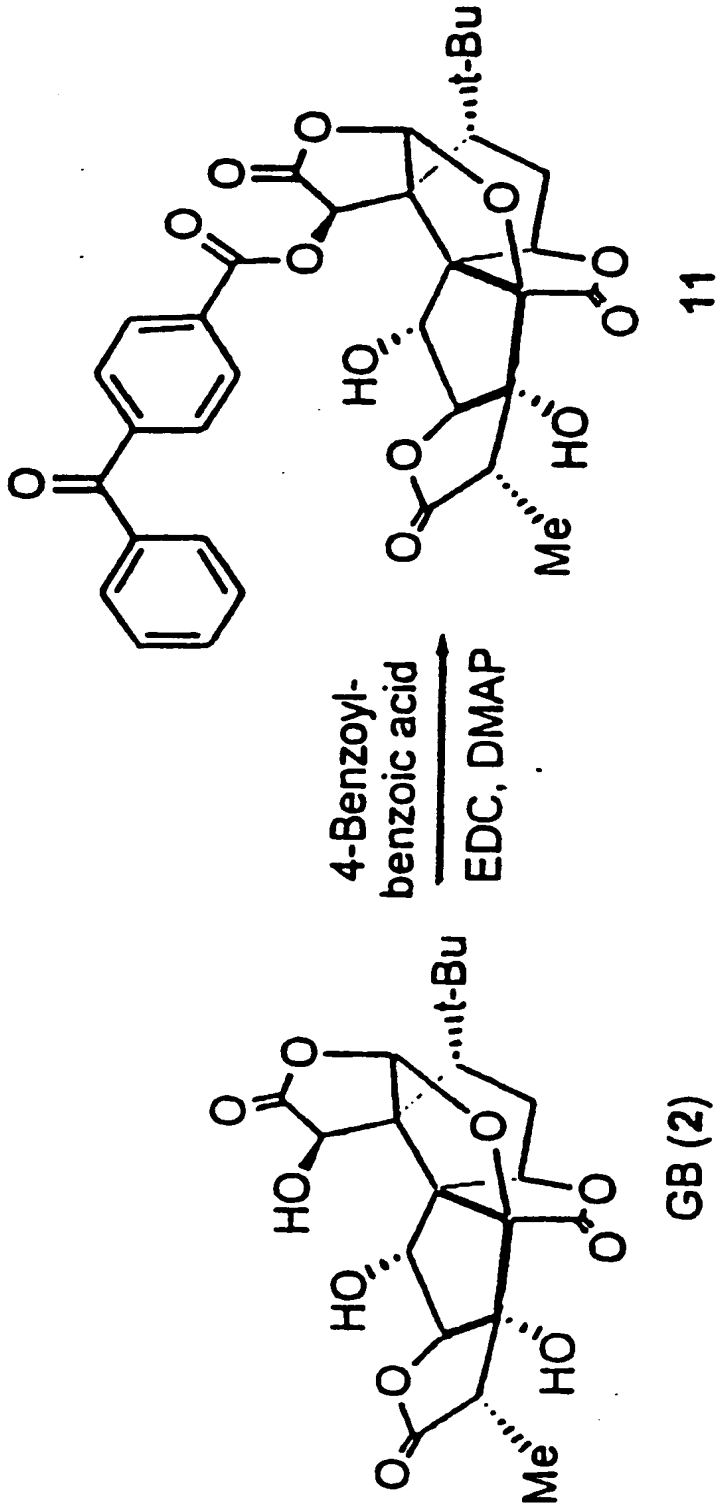


FIGURE 6

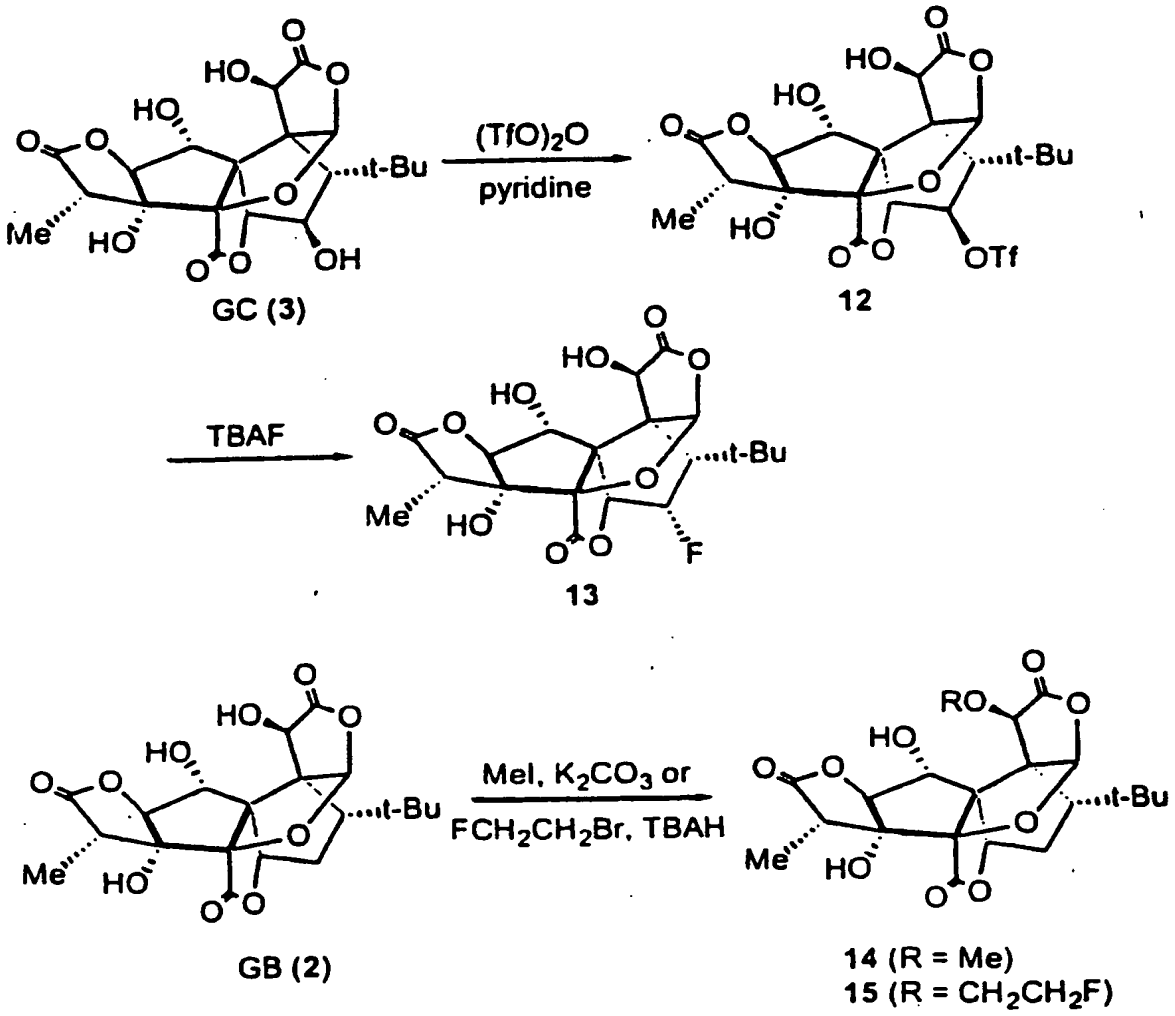

HO
HO
O
O
O
t-Bu
Me
HO
O
O
OH
GC (3)
(TfO)2O
pyridine
HO
HO
O
O
O
t-Bu
Me
HO
O
O
OTf
12
TBAF
HO
HO
O
O
O
t-Bu
Me
HO
O
O
F
13
HO
HO
O
O
O
t-Bu
Me
HO
O
O
GB (2)
MeI, K2CO3 or
FCH2CH2Br, TBAH
RO
HO
O
O
O
t-Bu
Me
HO
O
O
14 (R = Me)
15 (R = CH2CH2F)

FIGURE 7

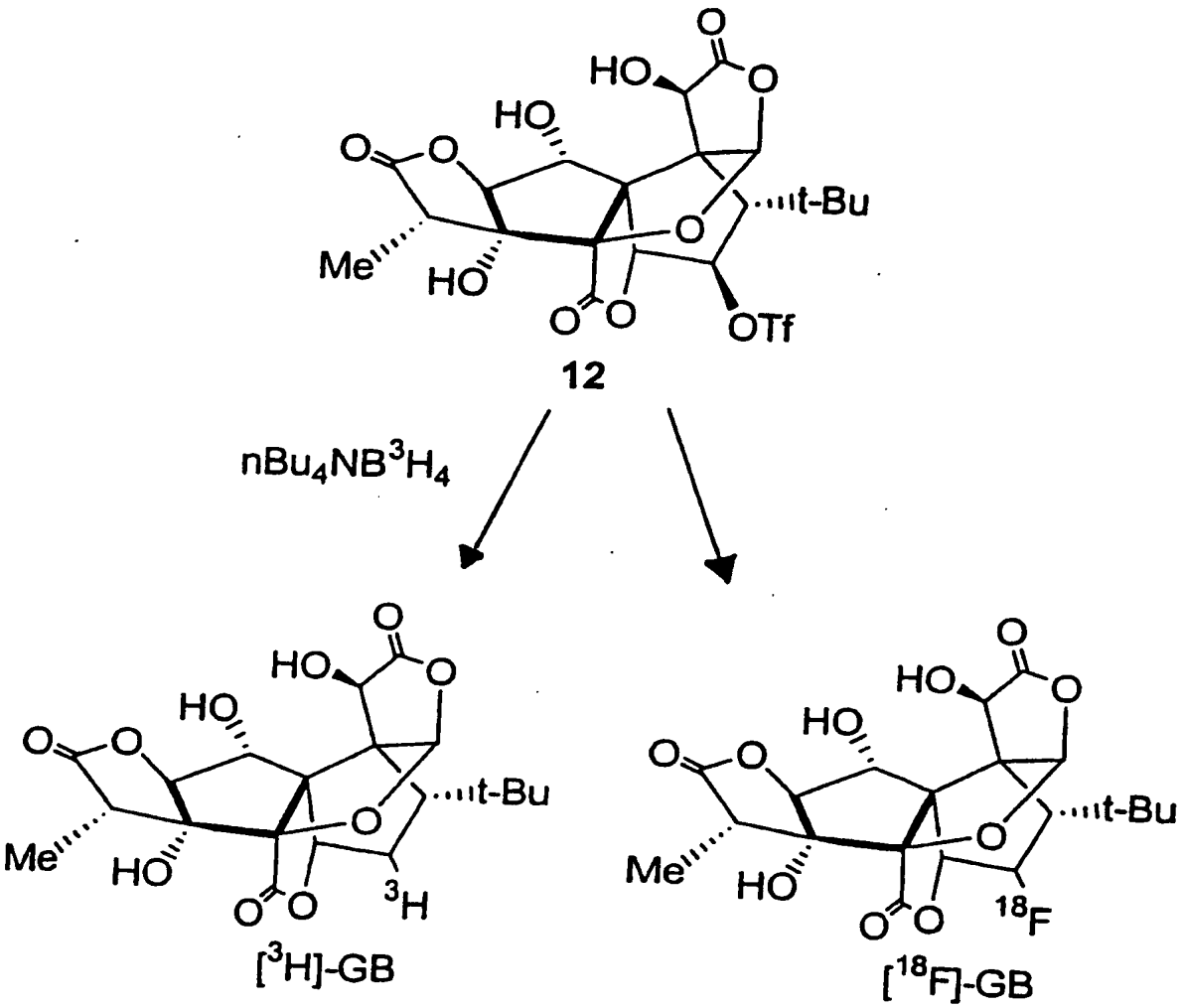
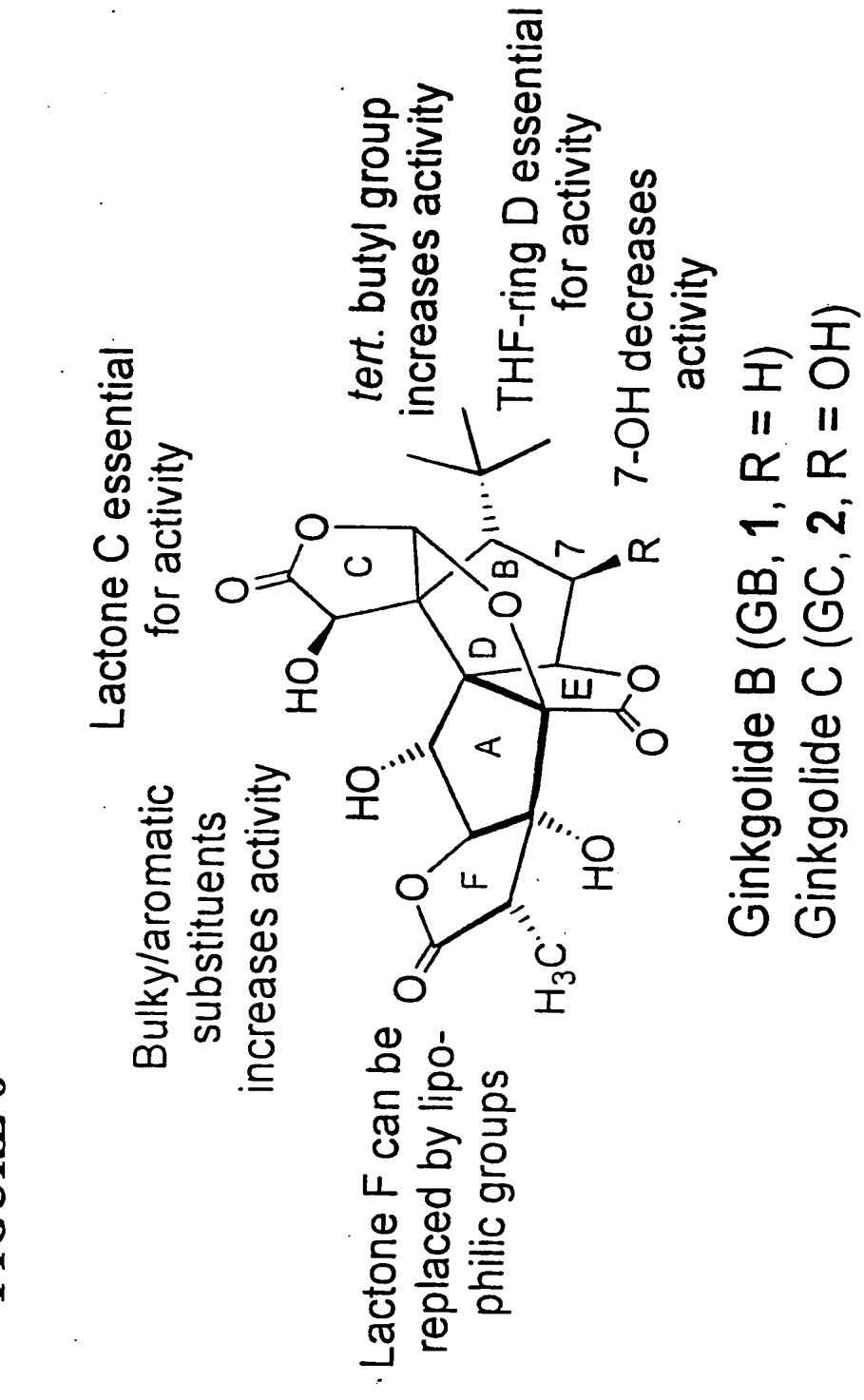

HO
O
O
HO
O
O
t-Bu
Me
HO
O
O
OTf
12
nBu4NB3H4
HO
O
O
HO
O
O
t-Bu
Me
HO
O
O
3H
[3H]-GB
HO
O
O
HO
O
O
t-Bu
Me
HO
O
O
18F
[18F]-GB

FIGURE 8


Lactone C essential for activity
Bulky/aromatic substituents increases activity
tert. butyl group increases activity
THF-ring D essential for activity
7-OH decreases activity
Lactone F can be replaced by lipo-philic groups
Ginkgolide B (GB, 1, R = H)
Ginkgolide C (GC, 2, R = OH)